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Contents

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A STUDY OF THE WINTER FEEDING HABITS OF THE SHORT-EARED OWL (*Asio flammeus*) IN THE TORONTO REGION[1]

BY A. W. F. BANFIELD[2]

Abstract

The winter feeding habits of the short-eared owl (*Asio flammeus* Pontoppidan) were studied during the period of 1936 to 1942 in the vicinity of Toronto, Ont. The owls used restricted roosts in several groups of Douglas fir (*Pseudotsuga taxifolia*) on a golf course. By means of frequent owl population censuses and collection of pellets, food requirements and correlations were calculated.

The winter incursions of the owls were found to be cyclic and to coincide with the local meadow vole (*Microtus pennsylvanicus*) cycle and to be independent of meteorological factors. Roosting behaviour was found to vary with snow cover.

From an analysis of 3000 pellets it was found that the meadow vole formed 82% of the food taken. The next animal most commonly preyed upon was the deer mouse (*Peromyscus leucopus*), which comprised 17% of the food, while birds formed 1%. No significant seasonal change in diet was noted over a period of five months.

It was calculated that a short-eared owl eats between 700 and 1600 mice per year. The average figure is thought to be nearer the minimum estimate. Translated to weight of mice this is equivalent to between 55.5 and 127 lb.

It was demonstrated that the amount of snow on the ground affected the relative availability of meadow voles and deer mice. This fact was reflected in changes in pressure on the populations of the two mice species due to owl predation.

The data presented pointed to the possibility that concentrations of avian predators could have an appreciable effect on local meadow vole populations. In the case under study it was estimated that winter short-eared owl predation might account for 10% of the vole population.

The data also suggested that during periods of deep snow, because of decreased availability of meadow voles, less food is taken and during open periods increased availability of these mice is reflected by increased food consumption.

Introduction

The short-eared owl (*Asio flammeus* Pontoppidan) has been one of the more favoured birds of prey because of its apparent preference for a diet of meadow voles (*Microtus* sp.). Where the population of these voles had increased to plague proportions, these owls have been noticed to congregate and to feed upon the voles. Such records are given by Adair (1) and Goddard (5) for vole plagues in Scottish border counties.

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Contribution from the Department of Zoology, University of Toronto, Toronto, Ont. This paper is based on a thesis submitted as partial requirement for the degree of Master of Arts in the University of Toronto, 1946.

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At Toronto, during the winter months for the past 10 years, there has been a good opportunity to observe the behaviour of these owls during periods of vole abundance. On Apr. 4, 1936, three short-eared owls were discovered roosting in a clump of Douglas fir (*Pseudotsuga taxifolia*) on York Downs golf course, immediately north of the city limits of Toronto. Each subsequent year the owls have returned in varying numbers to winter at the same locality.

Snyder and Hope (10) recorded the original discovery of the owls, and the analysis of about one thousand pellets that were picked up from beneath the perches. They correlated the appearance of the owls with a generally conceded local peak in the population of meadow voles (*Microtus pennsylvanicus*) during the winter 1935-36.

The following investigation was carried out over a period of seven years with a view to obtaining quantitative data on the food consumption of the owls in the wild state; the effect of certain environmental factors upon feeding habits; and the predator–prey relationship between the owls and the local mouse population.

Throughout the remainder of the paper, the name meadow vole or vole refers to *Microtus pennsylvanicus*, while the term deer mouse refers to either *Peromyscus leucopus noveboracensis* or *Peromyscus maniculatus bairdii*. Both these forms occur at Toronto. *Peromyscus leucopus* is the more common and more general in its habitat choice. *Peromyscus maniculatus bairdii* shows a preference for open wastelands or sandy situations. The great majority of skulls examined from the owl pellets were referable to *Peromyscus leucopus* but it is possible that a few skulls representing the other form were overlooked. The skulls of this genus are usually found crushed in the pellets, which makes identification more difficult.

Location and Habitat

The roosting area used by the owls was of limited extent. They frequented seven clumps of ornamental conifers planted between fairways. Each clump consisted of about a dozen trees, 15 to 20 ft. high, 3 to 6 yd. apart. These clumps were placed about 25 yd. apart along the edges of three fairways. The total area in which these clumps were scattered was about 10 acres. The plantings consisted of Douglas fir (*Pseudotsuga taxifolia*) with a few white cedar (*Thuja occidentalis*) and tamarack (*Larix laricina*) included in the groups. The owls used only the Douglas firs as roosts, probably because they offered denser cover.

Flanking the site on three sides, were deep wooded ravines draining into the Don river valley to the east. On the western side was a waste meadowland of about two hundred acres, dotted with a few cattail marshes surrounded by willows (*Salix* sp.), scattered white elms (*Ulmus americana*), and a few small cottages. This area was separated by a nursery and woodlot from the more extensive uncultivated grassland known as Armour Heights. This

whole area was subject only to occasional haying, and controlled spasmodically by local grass fires in spring. It supported an abundant grass and herb vegetation, which proved an admirable habitat for meadow voles.

Method of Study

The area was visited as regularly as possible throughout the residence period of the owls. The site was also visited at different times of day to observe the owls under different daylight conditions. These visits averaged five days apart over the season. All the clumps were approached; the owls were flushed and counted; then observed in flight. The disgorged pellets beneath the roosts were then collected. Pellets are the indigestible remains of a meal, such as fur, feathers, and bones. They are regurgitated in the form of compact balls. Their analysis gives data on the food consumed by the owls. Later in the laboratory these pellets were counted and analysed.

Roosting Behaviour

Early in the study it was noted that when the ground was bare of snow the owls were found roosting on the ground, in the fields. When there was snow on the ground, the owls were found roosting in the Douglas fir clumps on the golf course.

While roosting in the fields, during the day, the owls utilized 'forms'—depressions in the grass clumps. Scattered about were pellets, feathers, fragments of mice, and excreta. These 'forms' were occupied by one or more owls and were usually grouped together. When flushed, several owls rose from a small area. The owls seldom occupied the same forms for two successive nights, although they might roost nearby again.

After a heavy snowfall with the fields and meadowland well covered and only the tips of the weed stalks projecting, the owls were observed roosting in the coniferous clumps on the adjoining golf course. If there were later protracted thaws and the fields again became bare, the owls returned to roosting on the bare ground in the fields.

Under the above described conditions it was found impossible to carry on any quantitative studies while the owls wandered about the fields during the early part of the winter season. But usually by January, under the influence of accumulating snow, the owls would congregate to roost in the conifer clumps during the daylight hours. The collection of quantitative data was then possible. This behaviour will be referred to later in connection with the owl population and illustrated by Figs. 1, 2, 3, 4.

It appeared that this roosting behaviour could be explained as a response to cover requirements. In a bare winter field these brown, mottled owls blended particularly well with the dried grass, and they were usually not seen until flushed from their 'forms'. A heavy fall of snow left the fields with a contrasting white background that was poor cover for the birds. Under these

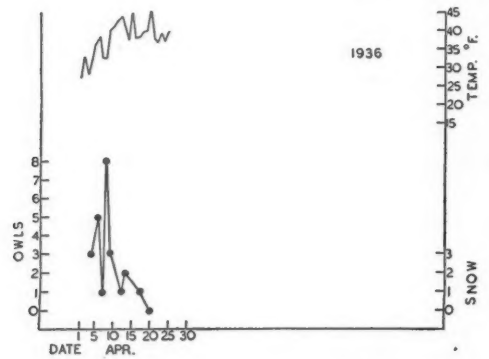

FIG. 1. *Owl population, snowfall, and daily mean temperatures for the winter 1935-6.*

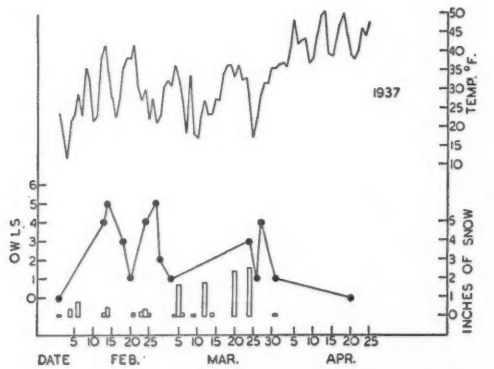

FIG. 2. *Owl population, snowfall, and daily mean temperature for the winter 1936-7.*

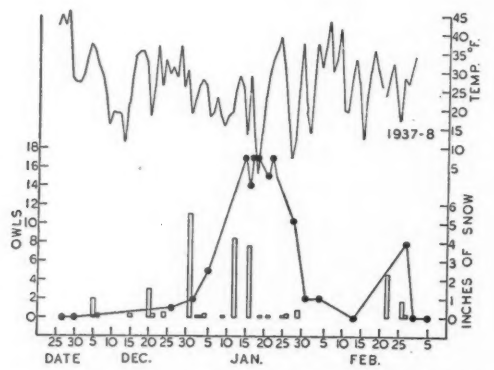

FIG. 3. *Owl population, snowfall, and daily mean temperature for the winter 1937-8.*

conditions the owls roosted in the dense cover of the evergreens. As they perched close to the trunks, they were again unnoticed until flushed.

Predators of the Short-eared Owl

During the winters that the owls were under observation they were comparatively free from molestation by man, since the area they frequented had few houses; the golf course was unused from October until April; and it was

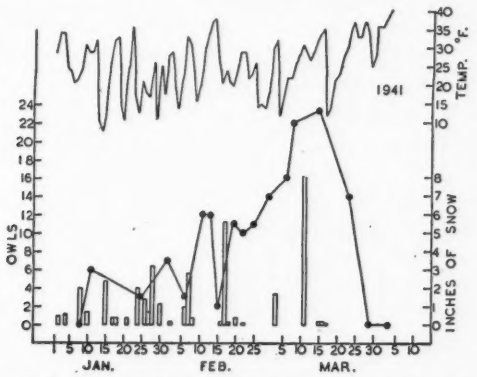

FIG. 4. *Owl populations, snowfall, and daily mean temperatures for the winter 1940-1.*

unlawful to carry firearms in the area. The above factors tended to give the owls some freedom of movement. During the study no complaints of owl predation on domestic animals were received from the local populace.

There were present in the area several predaceous animals that might be considered as competitors of the owls, including:

Domestic cat (*Felis* sp.)—a few were seen.

Skunk (*Mephitis mephitis*)—numerous winter tracks and two occupied burrows were found in the owls' hunting range.

Weasel (*Mustela frenata*)—a few tracks were seen.

Marsh hawk (*Circus hudsonicus*)—on two occasions during November and December these hawks were seen to pursue short-eared owls for short distances. On the other hand, Bent (2) gives observations of the short-eared owl pursuing marsh hawks. It is unlikely that these hawks are active predators of the short-eared owl under these conditions.

Great horned owl (*Bubo virginianus*)—observed in the nearby wooded ravines on several occasions.

Goshawk (*Astur atricapillus*)—one was observed flying over the golf course on Dec. 26, 1937.

The only remains of a short-eared owl observed were found in a small ravine on the golf course on Jan. 4, 1938. They gave every indication of a hawk or owl kill, possibly the previously mentioned goshawk.

Yearly Variations in Owl Populations

The owl population during each winter period may be indicated by two methods. The first is by direct observation and calculation of the total number of owl days. One owl day is the total amount of foraging by one owl in a 24 hr. period. The second method of indication is given by the total number of pellets collected from beneath the roosts during the complete winter season. This second method is only roughly indicative of the owl population. There is a secondary significance to this figure, as it also reflects the mouse population.

Returning to the first method, the numbers of short-eared owls flushed from the roosts on the golf course on each observation date are shown on Figs. 1, 2, 3, 4, for the winters 1935-6, 1936-7, 1937-8, and 1940-1, respectively. And on the same figures are shown the mean daily temperatures in ° F. and daily amounts of snowfall in inches. The roosts were not discovered until April, 1936, but there was a previous record of 22 owls 'during the winter'. This explains the great accumulation of pellets collected. During the winters of 1938-39 and 1939-40 the owls did not take up permanent roosts on the golf course but appeared irregularly about the area in comparatively small numbers. Under these latter conditions no quantitative studies were possible.

The peaks in the owl population curves tend to show the local wintering owl population. The dips in the owl curves indicate prolonged mild or snowless periods when the majority of the owls left the conifer roosts and returned to roost in the fields. This phenomenon has been considered previously in the paper.

Fig. 5 shows the total number of owl days for each season and the number of pellets collected from beneath the roosts. Had the study been begun in December, 1935, it is expected that the number of owl days would have reached that of 1940-1. The number of pellets collected shows a marked periodicity with peaks during the winters of 1935-6 and 1940-1.

Fig. 6 shows the number of pellets with two sets of meteorological data, snowfall, and number of day degrees, which are a summation of the differences between the daily mean temperature and 65° F. This gives a measure of the severity of the winter: the greater the total day degrees, the colder the winter.

It is apparent that there is no correlation between these meteorological factors and the winter owl populations. We must look elsewhere for an explanation of their periodic occurrence.

Snyder and Hope (10) presented data to show that locally there had been a peak in numbers of the meadow voles coincident with the influx of short-eared owls during the winter of 1935-6.

During the succeeding winters the numbers of meadow voles decreased locally. And during these winters short-eared owls were present in lesser numbers as indicated on previous figures. No great damage to trees or crops was noted. But during the winter of 1940-1 the population of voles in the uncultivated grassland again assumed plague proportions. A reconnaissance

across Armour Heights during the winter showed the fields honeycombed with vole runways and numerous grass nests placed on the average of 3 to 10 yd. apart. Meadow voles were easily caught by hand in haystacks and under debris. On the local roads cars exacted a high mortality on the voles. By spring many small trees and shrubs had been debarked or girdled. During that winter once again there was a heavy influx of owls as indicated by the number of pellets collected during the winter of 1940-41.

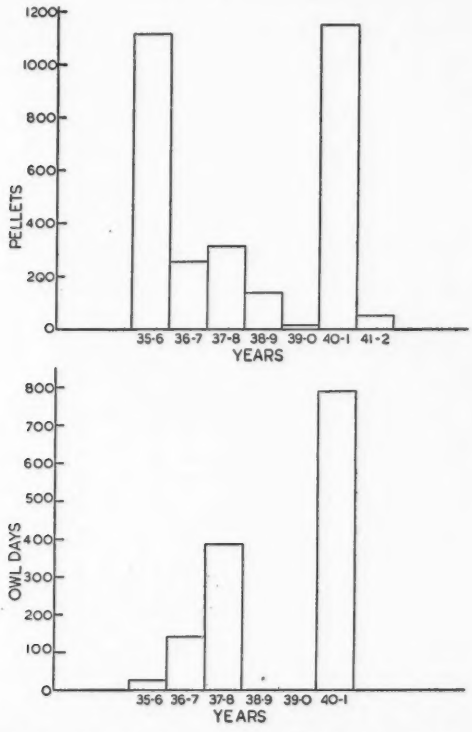

FIG. 5. *The total owl days and the total pellets collected from beneath the roosts for all the winters under study.*

The following winter voles were visibly less numerous and short-eared owls were transitory about the golf course. During the winter of 1945-6 observers stated that voles were abundant in the fields of York Downs and the short-eared owls were present in even greater numbers than before. On Jan. 28, 1946, 36 owls were flushed from the evergreens on the golf course.

Although no quantitative data were collected on vole populations at the locality during the study, it seems safe to assume that locally the vole population showed a marked periodicity with peaks during the winters of 1935-6, 1940-1, and 1945-46. This is based on the observations of several naturalists.

From the present study, with seven winters' data at hand, it appears that the winter incursions of short-eared owls in the Toronto region show a periodicity coincident with the local meadow vole cycle. This, then, is an example of a predator congregating at the locality of its prey's population peak, over a

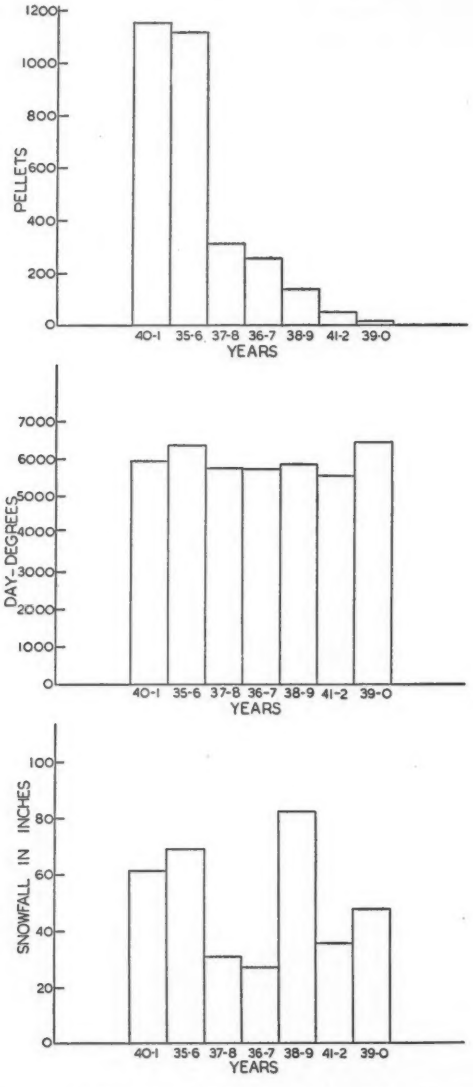

FIG. 6. *Comparison of pellets collected per year, total snowfall, and total day degrees for all years under study.*

period of years; and the predator species therefore showing a local periodicity coincident with its prey's population cycle.

Hamilton (7) believed that there was a regular four-year cycle in the meadow vole populations of New York state. He based this conclusion on an exhaustive population study and a collation of population data from other observers. His last reported peak coincided with the local Toronto peak in 1936. But locally at Toronto, from purely qualitative observations, it appeared that the last two vole population peaks showed a five year interval. The fact that the area is a more or less uncultivated island may mean that it should not be assumed to be typical.

Pellet Analysis

The analysis of the contents of all the pellets collected from beneath the roosts on the golf course for the six years 1935-41, is shown in Table I. Results of 1935-6 and 1936-7 are taken from Snyder and Hope (10).

TABLE I

PELLET ANALYSIS BY YEAR

Years		1935-6	1936-7	1937-8	1938-9	1939-40	1940-1	Total
Pellets		1078	252	311	136	12	1145	2934
Animals		1647	415	405	246	19	1666	4398
Microtus	No.	1181	389	371	168	18	1475	3602
	%	71.7	93.7	91.8	68.4	94.8	88.6	81.8
Peromyscus	No.	450	22	30	73	1	185	761
	%	27.3	5.3	7.5	29.6	5.2	11.1	17.5
Mus	No.	1	—	—	2	—	1	4
	%	0.1	—	—	0.8	—	0.0	0.1
Plectrophenax	No.	8	2	2	2	—	2	16
	%	0.5	0.5	0.5	0.8	—	0.1	0.3
Spizella	No.	—	—	—	—	—	3	3
	%	—	—	—	—	—	0.2	0.1
Otocoris	No.	1	1	—	1	—	—	3
	%	0.1	0.2	—	0.4	—	—	0.1
Passer	No.	2	—	—	—	—	—	2
	%	0.1	—	—	—	—	—	0.0
'Birds'	No.	4	1	—	1	—	—	6
	%	0.2	0.3	—	0.2	—	—	0.1

It is evident from this table that the meadow vole and deer mouse form the basis of diet for the short-eared owl under these conditions and that all other animals taken are incidental. The average percentages of voles and deer mice based on three thousand pellets over six years is 81.8% meadow voles and 17.3% deer mice. For four of the six years the percentage of vole to deer mice remained fairly constantly above this figure. During the winters of 1935-6 and 1938-9, the percentage of deer mice rose to about 30%. In this

study only the population cycle of the meadow vole has been considered. A cycle in the population of deer mice is quite possible but usually less evident. It is suggested that such a cycle probably affected the availability of deer mice over field voles during these two years. A second factor, which is climatic, will be considered later.

The percentage of birds taken averaged about 0.7% and varied from 0 to 1.2%. This shows that birds are seldom taken under the conditions of the present study. The birds, tree sparrow (*Spizella arborea*), snow bunting (*Plectrophenax nevalis*), horned lark (*Otocoris alpestris*), and house sparrow (*Passer domesticus*) are inhabitants of fields, which are the normal hunting ground of the short-eared owls.

TABLE II

PELLET ANALYSIS BY MONTH, DATA FOR ALL YEARS INCLUDED

Month	Pellets	*Microtus*		*Peromyscus*		*Mus*		Birds		Total
		No.	%	No.	%	No.	%	No.	%	
Nov.	8	7	87.7	0	0.0	1	12.3	0	0.0	8
Dec.	70	61	41.2	82	55.4	0	0.0	5	3.4	148
Jan.	333	387	90.7	37	8.5	0	0.0	3	0.8	427
Feb.	458	642	89.1	66	10.4	0	0.0	3	0.5	721
Mar.	697	978	96.4	33	3.4	1	0.1	1	0.1	1013
Apr.	166	200	93.0	15	7.0	0	0.0	0	0.0	215
Totals	1732	2275	89.9	233	9.4	2	0.2	12	0.5	2532

In Table II the pellets examined are arranged according to the month in which they were collected, in order to show any monthly trends in diet. Voles predominate in every month and their percentage fluctuates around 90% except for December, then there was an evident rise in the percentage of deer mice and a decrease in percentage of voles. Not too much significance should be attached to this, on a monthly diet basis, as the majority of pellets examined for this month came from one lot, which was associated with a period of heavy snowfall. This fact will be considered later.

The percentage of birds taken decreases from December to April. This might reflect the decreasing availability of birds during the late winter when the population is low before the migration influx. But in view of the small percentage under consideration, it appears unlikely that this is of significance. In general it is concluded that there was no appreciable change in diet during the winter months.

Fig. 7 shows the results of the analysis of 1361 pellets collected from 1937 to 1941 based on the number of animals per pellet. It is shown that 55.8% of pellets examined contained one meadow vole and an additional 29.4% contained two meadow voles. Chitty (3) working with a captive short-eared owl has shown that a pellet represents one meal and that it is disgorged before another meal is taken. He also observed that a pellet was held on the average

five to seven hours before being ejected. It may be assumed that in the wild state, one pellet represents one forage, because all the contents are in the same state of digestion. From the graph it is noted that in 85% of the forages one or two meadow voles were captured. The average number of deer mice per

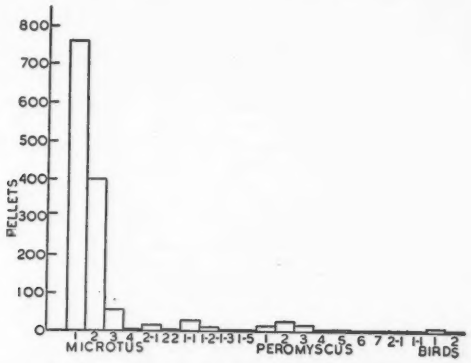

FIG. 7. *The number of animals per pellet.*

forage was two or three. The deer mouse being smaller than the meadow vole, it takes more of them to satisfy the food requirements of the owls. Finally although birds form an insignificant percentage of the diet, one bird per forage occurred in 60% of the pellets containing birds, suggesting equal food value to the meadow voles.

From these considerations the maximum food requirements for a short-eared owl may be estimated. From Table I the average number of animals per pellet (forage) is 1.5 approximately. From field observations and the data of Chitty, the number of forages per day would be two to four—at dawn and dusk for certain, possibly at midday and midnight. If we assume an average of three forages per day, in one year 1640 mice would be captured. This is based on optimum considerations and would tend to be a maximum estimate.

Food Consumption Calculations

By counting the squares under the curves, in Figs. 1, 2, 3, 4, the amount of hunting carried out by the owls using the roosts between dates when pellets were collected can be calculated in terms of owl days. By multiplying the number of owl days by the number of pellets collected for the same period, an estimate is reached for the number of pellets disgorged per owl for that period. Finally by multiplying this figure by a figure representing the contents of the pellets examined, an estimate of the food consumed by an owl during that period is arrived at. An analysis of the data for food consumption for the four winters, 1935-6, 1936-7, 1937-8, and 1940-1 is shown in Tables III, IV, V, and VI, respectively. The ratio of pellets to owl days gives a measure of the number of forages. The ratio of skulls to pellets

TABLE III

PELLET AND POPULATION ANALYSIS, 1935-6

Date	Owls	Owl days	Pellets	Pellets / Owl days	Skulls	Skulls / Pellets	Skulls / Owl days	*Microtus*	*Peromyscus*	*Peromyscus*, %
Apr. 7	1	0	1078		1647	1.53				27.3
" 8	8									
" 9	3	9.1	18	1.99	24	1.33	2.62	19	5	21.0
" 12	1									
" 13	2	7.5	12	1.60	13	1.08	1.73	12	1	7.5
" 17	1	6.0	1	0.17	3	3.00	0.50	3	0	0.0
" 20	0	1.5	0							
Total		24.5	31		40					
Av.				1.25		1.29	1.66			

TABLE IV

PELLET AND POPULATION ANALYSIS, 1936-7

Date	Owls	Owl days	Pellets	Pellets / Owl days	Skulls	Skulls / Pellets	Skulls / Owl days	*Microtus*	*Peromyscus*	*Mus*	Birds	*Peromyscus*, %
Feb. 1	0	0	0									
" 13	4	18	36	2.00	65	1.72	3.47	60	5			8.1
" 14	5											
" 18	3	18.6	132	7.12	219	1.66	12.3	204	13	0	2	5.9
" 21	1											
" 24	4	13.2	3	0.23	6	2.00	0.45	3	2	0	1	33.3
" 27	5											
" 28	2	16.8	9	0.54	17	1.89	1.01	17	0	0	0	0.0
Mar. 3	1	4.4	1	0.23	1	1.00	0.23	1	0	0	0	0.0
" 24	3	43.6	48	1.10	70	1.46	1.61	69	0	0	1	0.0
" 26	1											
" 27	4											
" 31	1											
Apr. 20	0	25.0	23	0.92	30	1.30	1.20	29	1	0	0	3.3
Total		139.6	252		415			380	21		4	
Av.				1.83		1.64	2.99					

remains fairly constant and usually reflects only the size of prey taken. The ratio of skulls to owl days gives a measure of food consumed by an individual owl over the period since the last observation. The percentage of deer mice taken reflects changes in diet as birds form an insignificant part.

From these tables, the number of mice consumed for an arbitrary period can be calculated from the average of skulls per owl days. For convenience the period chosen is a year.

TABLE V

PELLET AND POPULATION ANALYSIS, 1937-8

Date	Owls	Owl days	Pellets	Pellets / Owl days	Skulls	Skulls / Pellets	Skulls / Owl days	*Microtus*	*Peromyscus*	Birds	*Peromyscus*, %
Nov. 27	0	0									
" 30	0										
Dec. 26	1	14	7	0.50	9	1.29	0.64	9			0.0
Jan. 2	2										
" 5	5										
" 15	17	128.4	47	0.36	71	1.51	0.55	61	10		14.0
" 16	14										
" 17	17										
" 18	17										
" 21	15	95.2	36	0.42	56	1.57	0.59	51	5		9.0
" 22	17										
" 23	15										
" 29	10	110.6	131	1.19	151	1.15	1.37	142	8	1	5.8
" 30	5										
" 31	2										
Feb. 4	2	19.0	78	4.11	97	1.24	5.11	89	6	2	5.8
" 12	0	7.2	7	0.97	10	1.43	1.43	10			0.0
" 27	8	13.4	5	0.40	10	2.00	0.82	9	1		10.0
Mar. 1	0		0								
Total		386.8	311		404			371	30	3	
Av.				0.81		1.30	1.04				

From 1935-6 data we get 617 animals per year.
1936-7 " " " 1100 " " "
1937-8 " " " 382 " " "
1940-1 " " " 704 " " "

The average based on four years' studies equals 701 animals per year. The study during the winter of 1940-1 was the most critical and its result is considered more reliable. It shows close agreement with the average figure of 701 mice per year. These calculations show a wide variation represented roughly by 700 $\pm$ 45% mice per year (birds are ignored as they form so small a part of the diet). Whether the wide variation in these estimates reflects corresponding differences in the availability of food due to different densities of owls and mice, or are largely due to experimental error cannot at present be determined.

Because of the possibility that all pellets ejected by the owls were not found, these figures give minimum estimates of food consumption. This loss will be considered under discussion. It will suffice to conclude here that one short-eared owl would eat at least 700 mice $\pm$ 45% based on its winter feeding habits.

To translate this figure to weight of mice, an average weight of 35.7 $\pm$ 0.6 gm. for *Microtus* and of 21.9 $\pm$ 0.7 gm. for *Peromyscus* has been assumed.

TABLE VI

PELLET AND POPULATION ANALYSIS, 1940-1

Date	Owls	Owl days	Pellets	Pellets / Owl days	Skulls	Skulls / Pellets	Skulls / Owl days	*Microtus*	*Peromyscus*	*Mus*	Birds	*Peromyscus*, %
Jan. 8	0	0	62		138	2.23		51	82	0	5	59.4
" 11	6											
" 25	3											
Feb. 1	7	105.7	41	0.39	52	1.27	0.50	44	8	0	0	15.4
" 6	3	24.6	28	1.14	36	1.29	1.46	33	3	0	0	8.4
" 11	12	35.5	45	1.27	60	1.34	1.70	57	3	0	0	5.0
" 13												
" 15	2	37.5	81	2.15	119	1.47	3.19	82	37	0	0	32.2
" 16	4											
" 19	11											
" 22	10	56.7	50	0.88	65	1.30	1.15	61	4	0	0	6.0
" 25	11	31.5	60	1.91	90	1.50	2.85	83	7	0	0	7.8
Mar. 1	14	50.5	29	0.58	44	1.52	0.88	43	1	0	0	2.0
" 6	16	75.0	95	1.30	141	1.49	1.89	138	3	0	0	2.2
" 8	22	38.0	95	2.50	124	1.30	3.26	121	2	1	0	1.6
" 15	23	157.5	182	1.15	280	1.54	1.78	264	16	0	0	5.5
" 22	14	129.0	150	1.16	200	1.33	1.55	194	6	0	0	3.0
" 29	0	48.0	114	2.38	157	1.38	3.27	152	5	0	0	3.0
" 30	0	0.0										
Apr. 3	0	0.0	10		15	1.50		15	0	0	0	0.0
Total		788.5	970		1521			1338	177	1	5	
Av.				1.23		1.57	1.93					

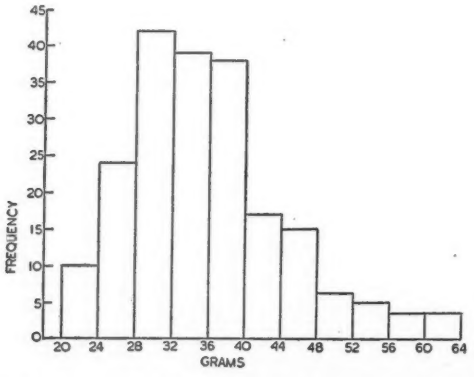

FIG. 8. *Histogram of the weights of 202 adult meadow voles.*

These weights are based on weights of adult Ontario specimens in the Royal Ontario Museum of Zoology. A histogram of the weights of 202 adult *Microtus* is shown in Fig. 8.

Assuming a standard diet of 90% meadow voles and 10% deer mice, it was calculated that a short-eared owl would eat at least 55.5 ± 45% lb. of mice per year, based on the figure of 700 mice per year.

Chitty (3) from feeding experiments on a captive short-eared owl in England concluded that in one year an owl would certainly eat more than 47 lb. of voles, probably more than 95 lb. but less than 142 lb. It is seen that his minimum estimate is close to the above calculation based on feeding under natural conditions. His owl, although mature, gained 12.5% of its original weight during the experiments. Such an increase would be unlikely with winter feeding under natural conditions.

Returning to our estimate of the maximum food requirements of one short-eared owl, of 1640 mice per year, this figure translated to weight gives 127 lb. This figure also shows close agreement with Chitty's data.

He translated this weight to 2000 ± 50% meadow voles per year. He was considering the European meadow vole (*Microtus agrestis*), which would average about 25 gm. in weight as compared to 35.7 gm. for the larger (*Microtus pennsylvanicus*).

Availability of *Peromyscus*

The two chief prey animals of the short-eared owl, the meadow vole and the deer mouse differ widely in their choice of habitat and terrain. The meadow vole is a denizen of meadows and grasslands, where it utilizes a wide ranging system of runways in the grass. It seldom leaves the security of the tunnels and a snowfall gives added protection. The deer mouse, on the other hand, is a forest mouse. It makes its home in thickets, brush piles, fence rows. The deer mouse is a wide ranging mouse whose tracks are commonly seen on the snow.

Hendrickson and Swan (8) working in central Iowa noted that as the temperature went down the percentage of *Peromyscus* in the pellets of short-eared owls increased. Vulnerability of *Microtus* seemed to increase as their tunnels were exposed by melting of the ice and snow. Deep snow serves as a protection for voles and puts the pressure on deer mice.

This relationship has been looked for in the present study. Fig. 9 shows the percentage of deer mice in the pellets plotted with the snowfall in inches and the daily mean temperature for the observations of 1937-8. The percentage of deer mice is plotted as a series of straight lines since this percentage refers to a group of pellets collected between two collecting dates. The snowfall in inches of snow is plotted on a daily basis during the period of observation: as is also the daily mean temperatures.

From a casual inspection of the graph it appears that there is a rough correlation between the percentage of deer mice and the snowfall amount—the heavier the snowfall the higher the percentage of deer mice in the pellets. The relationship is not strict because of the high experimental error. There was a strong possibility of pellets being buried in the snow and not retrieved

until later. Actually we are interested in the snow on the ground rather than the snowfall. The relationship between snowfall and lying snow is a complex one. Such factors as daily temperatures, cloudiness, evaporation, must be considered, so that a rigid correlation analysis is beyond the scope of this

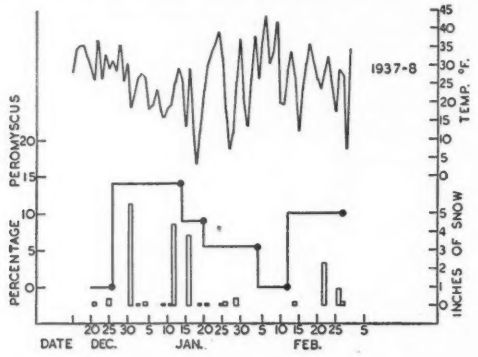

FIG. 9. *Comparison of percentage deer mice, snowfall, and daily mean temperature for 1937-8 records.*

study. A test of significance of the correlation between snowfall and percentage of deer mice would give a measure of the significance that snowfall plays in the availability of voles and deer mice.

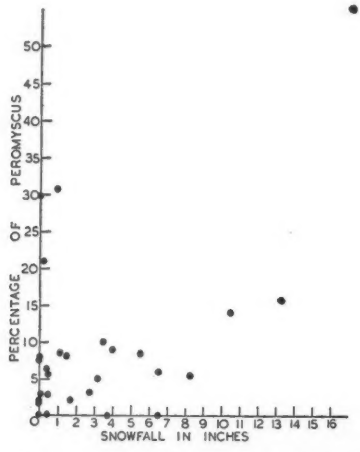

FIG. 10. *Percentage of deer mice plotted against snowfall—total data included.* $r = +0.625$; $t = 5.52$; $P < .001$.

Such a test of significance of the correlation was carried out. Fig. 10 shows a plotting of percentage of deer mice against snowfall for the period to which the pellets refer. The graph contains the complete data from the four winter's data presented in Figs. 1, 2, 3, 4, 5, 6.

There is a rough correlation apparent between snowfall and the percentage of deer mice. Standard statistical procedures were applied to these data. The coefficient of correlation between the snowfall and the percentage of deer mice in the pellets was found to be 0.625. The significance of this coefficient was tested by means of 'Students' t test; t was calculated to be 5.521. From Fisher's tables it was found that one would not expect a coefficient of correlation of such a magnitude in more than one out of 1000 chances from a random uncorrelated population. So we conclude that there is significant correlation between percentage of deer mice in the pellets and the amount of snowfall during the period. No significant correlation between percentage of deer mice in the pellets and average daily mean temperature was found.

We may conclude that snowfall affects the availability of meadow voles and deer mice. During periods of heavy snow meadow voles are protected by a blanket of snow. The pressure of predation by short-eared owls is then transferred to the deer mice, which are more available to the owl.

Animals Eaten per Day

It is the generally held opinion, based on physiological requirements, that, in order to maintain their heat requirements, active homoiothermic animals must eat more during cold weather than warm weather. This concept does not take into account the normal environmental condition in which an animal is usually situated.

In Canada periods of low temperature are usually associated with conditions of snow, frozen ponds, perhaps hard packed snow. To such an animal as the short-eared owl, these periods are times when natural food in the form of meadow voles is less available. Under these conditions it is unlikely that the owls are able to eat more to maintain their heat requirements. It is more likely that they have to eat heavily when food is plentiful during mild snowless periods and tide themselves over periods with cold frozen snow covered landscapes. It is during these periods of deep snow and low temperatures that one often finds animals dead from starvation or exposure.

From our general analysis Tables II, III, IV, V, we have a series of figures giving the skulls per owl day. These figures give a measure of the average number of animals captured by the owls over the previous period. From inspection this figure is found to vary widely, changing climatic factors would be one plausible reason why the owls ate more during certain periods than others. Fig. 11 shows the average number of animals eaten per day for the pellet period, plotted for the season, 1940-1, and plotted against this figure are the amount of snowfall and daily mean temperatures.

A rough correlation between animals per day and the climatic factors is evident. The greatest number of animals eaten per day occur when the weather has been mild and the snow melting, or the ground bare. The lower numbers of animals eaten were associated with cold spells and deep snow, the relationship of all the factors is exceedingly complex, and its analysis difficult.

A correlation between animals per day and snowfall would support the hypothesis that hunting was more successful with the vole runways exposed by melting snows and mild temperatures. During these periods the owls

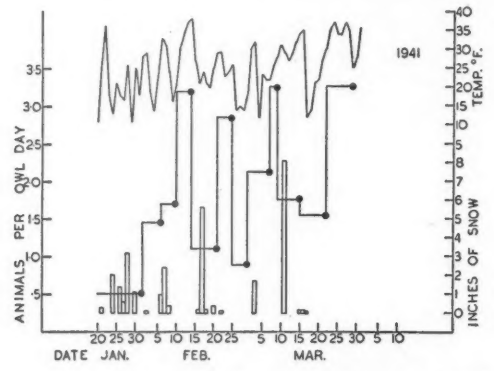

FIG. 11. *Number of animals eaten per day plotted against snowfall for period, 1940-1 date.*

were able to feed heavily and tide themselves over the periods when foraging was difficult in cold spells or heavy snowfalls.

Fig. 12 shows a plotting of animals per day against snowfall for all the observations over all the years studied. There is a suggestion of correlation

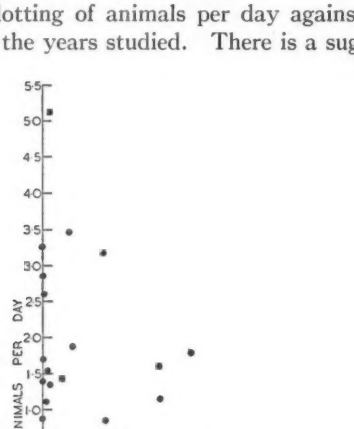

FIG. 12. *Animals eaten per day plotted against snowfall during period—total data. $r = -0.2041$; $t = 1.8$; $P = <0.1$.*

between animals per day and snowfall. A calculation of the coefficient of correlation between animals per day and snowfall was found to give an r equal to $-.2041$. The negative coefficient signifies that a rise in the amount

is accompanied by a decrease in animals per pellet. When this '*r*' was tested by means of 'Students' *t*, it was found that an *r* of this magnitude would be expected in about one out of 15 random samples from an uncorrelated population. As this is about the rejection point, this simple correlation between snowfall and animals per day is not considered significant, and the test has failed to prove conclusively our thesis. One of the factors that obscures the relationship is the fact that the owls consume more deer mice than meadow voles per forage. During periods of snowfall deer mice are more available than voles. These two factors would combine to give more skulls per day during periods of heavy snowfall, and thus obscure the correlation under consideration.

Although the test of correlation fails to support the hypothesis with certainty it is still thought worthy of consideration.

Predation Effect

The territory over which the owls hunted was carefully observed during daylight hours and at dusk. It appeared restricted to the uncultivated meadowland to the west of the golf course. This area was estimated by rough measurement to be approximately 200 acres. During the winter of 1940-1, the owls were present at York Downs in considerable numbers from Nov. 15 to Apr. 15. Referring to graph Number 4 it seems reasonable to assume a local population of 20 owls. During that same winter period their predation on meadow voles was at least,

$$700 \times \frac{5}{12} \times 20 \times \frac{9}{10} = 5250 \text{ voles.}$$

From this study no figures on meadow vole populations were obtained, but Hamilton (7) gave a vole population of 50 to 250 per acre (the latter figure for peak years). Assuming 250 voles per acre, a population of 50,000 meadow voles was estimated on the hunting grounds. The short-eared owls' predation would then account for at least 10.5% of the population. These figures are conservative estimates based on a minimum food requirements estimate. They suggest that the predation of these owls would have an appreciable effect on the vole population.

The short-eared owl was only one of several active predators that preyed on the voles. Others present were snowy owl (*Nyctea nyctea*), horned owl (*Bubo virginianus*), red-tailed hawk (*Buteo borelis*), and American rough-legged hawk (*Buteo lagopus*). These avian predators alone could conceivably exact up to 20% mortality on the vole population, and occurring at a time when reproduction was at a minimum, this predation might be an important factor in controlling the local vole population.

Elton (4) has shown from the works of Russian ecologists that they favour the theory that predators exercise control on rodent population peaks brought about by optimum food conditions. This theory is expounded by Kalabukhov (9) and is supported by an accumulation of ecological studies along lines

similar to the present study. There have been population studies on voles by means of metal tagging. These tags have been retrieved from the pellets of predaceous birds. There are also studies on pellet analysis from standard perches. All these studies suggest that the role of predators in controlling rodent populations may be greater than is usually accepted by American ecologists.

Discussion

Naturally these calculations are subject to considerable error. The chief source of error arises from the possibility that all the pellets disgorged by the owls during the period were not collected. With reference to this possibility it may be said that the roosts were so restricted that probably all the pellets disgorged by the owls at the roosts were collected. One must, however, consider the possibility of other roosts.

Guerin (6), working with the barn owl (*Tyto alba*) in France, established the fact that there were at least two daily ejections of pellets—"pelote nocturne and pelote diurne". The former followed a meal taken perhaps within three minutes from the beginning of the night's hunting. It was produced at the "station nocturne" before the owl took its second meal (about 5 a.m.) in July and returned to roost at the "station diurne" where the second pellet was produced. No similar behaviour has been described for the short-eared owl. According to Bent (2) short-eared owls hunt at dawn, dusk, and spasmodically throughout the day. In the present study, any pellets disgorged from the dawn forage would be at the "station diurne"—in this case the ornamental fir clumps. The pellets from any diurnal forages would also be deposited here. Unless the owls returned to roost at night in the evergreens the pellets from the dusk forage would be dropped elsewhere.

The hunting grounds were intensively covered, and no evidence of a "station nocturne" was found. A quantitative analysis of food consumption based on pellets collected from the "station diurne" tends to give a minimum estimate. At present it is impossible to assess the loss in pellets from the dusk forage. It could theoretically amount to 50%, if there were two pellet stations.

A second source of error from the possibility of shifting owl population between observations must be considered. These shifts were equally probable in either direction and therefore would have a cancelling effect.

There is one consideration that favours this method of study. It is that observation on large groups of animals over long periods of time, and under natural conditions would tend to give more natural results than experimentation on individuals in captivity.

Acknowledgments

I wish to express my appreciation of the assistance and advice given during this study by Prof. J. R. Dymond, Prof. A. F. Coventry, and Dr. J. MacArthur of the Department of Zoology of the University of Toronto.

I am indebted to the staff of the Royal Ontario Museum of Zoology for the use of the museum files and collections. I wish to acknowledge the friendly co-operation of Mr. C. E. Hope who examined about 50% of the pellets analysed in the study, including the data previously reported by Snyder and Hope (10). Mr. J. L. Baillie was of assistance in bringing to notice observations of other naturalists. Mr. S. C. Downing made available the mammal collection file for a compilation of weights.

Meteorological data were supplied by the Toronto Meteorological Office, Department of Transport.

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SOME EFFECTS OF TEMPERATURE ON THE PRODUCTION AND ELIMINATION OF DIAPAUSE IN THE WHEAT STEM SAWFLY, *CEPHUS CINCTUS* NORT.[1]

By R. W. Salt[2]

Abstract

Fully grown *Cephus cinctus* larvae go into an obligatory diapause, the ending of which involves the elimination of two separate factors. The first, or *x* factor, is eliminated only at subdevelopmental temperatures and has a positive temperature coefficient in a temperature range from the undercooling point up to the developmental threshold. The second, or *y* factor, is eliminated at either low or moderate temperatures, having a positive coefficient in a temperature range from the undercooling point up to about 30° C. When the *x* factor reaction is complete, diapause may be said to be 'broken', but it is not 'eliminated' until the *y* factor reaction is complete.

When the *x* factor reaction is complete but the *y* factor is still present, the insect can be returned to diapause by either high temperatures or a lack of adequate moisture or a combination of these. This sometimes occurs in nature, resulting in a two-year life cycle. Once the *y* factor has been eliminated the insect starts postdiapause development and can no longer return to a state of diapause.

Postdiapause development under constant favourable conditions is rather uniform after it has once started, but the time of its initiation is variable, dependent on the *y* factor. Evidence is presented that indicates that the *y* factor is eliminated faster in large larvae than in smaller ones, accounting for the rather wide variability in development in samples uniformly treated but not selected as to size.

At 10° C. roughly 40 to 110 days were required to break diapause, each larva requiring a definite conditioning period. Thus the process ends abruptly in individuals, gradually in a group. Under natural conditions diapause was broken as early as Oct. 19, 1945, in a few cases, and was broken in all cases by the end of January 1946.

Introduction

The wheat stem sawfly, *Cephus cinctus* Nort., is a univoltine species with an obligatory diapause in the mature larval stage. It reaches this stage in the late summer, and the diapause, which prevents further development in the fall when food and temperature conditions are unfavourable, is eliminated by the time spring temperatures rise sufficiently to allow development. The occurrence and elimination of diapause during the fall and winter is so universal that it would be of mere academic interest were it not for the fact that diapause can be reinstated in the spring. Farstad (2) has found that this "spring diapause" may affect large percentages of the larval population under drought conditions or under similar conditions created in stubble fields where shallow tillage operations have raised the infested stubs* to the soil surface. Small

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**The term stub refers to the short stubble that remains after the larva cuts the grain stem, about the time the grain is ripening. The stem is weakened at the cut, made just above the soil surface, and readily breaks off. The larva plugs the end of the stub with frass and lines the entire cavity with a cocoon. It spends the fall, winter, and spring in the stub, changing to pupa and adult before emerging through the frass plug in June.*

numbers of larvae in spring diapause are commonly found throughout western Canada as a result of localized drought conditions in their microclimates. Soil erosion by wind or water, whether on a large or small scale, tends to expose stubs to subsequent heat and desiccation. The chances of larvae in such stubs re-entering diapause are much greater than those in stubs with the normal protection of the surrounding soil. Thus a study of diapause in *Cephus cinctus* is of practical importance.

The literature on diapause in insects is so voluminous that no review of it will be attempted here. Reviews are contained in other papers, a recent one being given by Prebble (3-7). The phenomenon of diapause is so widespread among insects that its manifestations are variable. It may occur in univoltine, bivoltine, or multivoltine species. It may occur in feeding or non-feeding stages, the latter being most common. It may be of long or short duration, and it may be produced and destroyed by a variety of factors.

Diapause in *Cephus Cinctus*

In *Cephus cinctus* diapause occurs only in the fully grown larva in the stub. By this time the larva has completed feeding, emptied the gut, and lined the stub with a cocoon. It has a characteristic S-shape, which persists until after diapause has been eliminated and development is resumed, when it straightens out to become a prepupa. No ecdysis accompanies this change, but as the larva becomes straight, constrictions appear between the head and thorax and between the thorax and abdomen, producing three distinct body regions. In this paper the two stages will be referred to as S-larva and prepupa, but it will be seen later that while they are morphologically distinct, important physiological changes occur that do not coincide exactly with the externally apparent morphological change. It is impossible, for example, to tell at a glance whether an S-larva is in or out of diapause. Under both conditions they wriggle actively, and it is only by the use of some physiological test that the distinction can be made, unless one waits until the prepupal stage is either reached (diapause broken) or fails to be reached (diapause intact). This latter method, however, is rather unsatisfactory, for it is not only time-consuming (several weeks may be required) but it also introduces the disadvantages of uncertain rearing techniques. For while, if an S-larva becomes prepupal at ordinary rearing conditions, it is certain that its diapause had been broken, on the other hand if it fails to become prepupal it is not certain that its diapause was not broken. It will be shown later that a larva in which diapause is broken may return to a state of diapause under certain adverse conditions. In experimental work such conditions were often difficult to avoid when it was necessary to remove the larvae from their stubs. Rearing methods are therefore of considerable importance.

Under natural field conditions diapause in *Cephus cinctus* is obligatory in that all larvae without exception are in diapause when they cut the stem in the fall. Similarly diapause is broken during the fall and winter in all

individuals. The finding of diapause larvae in the spring and summer indicates a return to diapause and not a failure of winter conditions to break diapause.

Materials and Methods

Infested stubs were either collected fresh from wheat stubble when required or taken from large collections made in the fall and stored for later use under a variety of conditions. Since the storage conditions are of great importance they are specified later for each experiment as it is described.

All stubs were collected from fields of Marquis or Red Bobs wheat, both highly susceptible varieties. Constant temperatures were maintained with reasonable accuracy in thermostatically controlled cabinets. Temperatures above room temperature were maintained within a range of about 2° C., while refrigerated cabinets varied within 4° or 5° C. The extremes of variation, however, were infrequent, and occupied only a small percentage of the time involved. If it had been possible to record them, the mean temperatures would undoubtedly have been close to the values listed, and the standard deviations less than 0.5° C.

Relative humidity was controlled by the use of sulphuric acid and water mixtures in glass containers. The air in these was not circulated and was disturbed as little as possible during its period of use.

Adequate moisture is required for development following the breaking of diapause. This applies particularly to the early stages of postdiapause development. Tolerance of moist conditions at this time appears to be quite wide, but dry conditions readily prevent the initiation of development. Unless otherwise stated, stubs used in this work were kept at all times in damp sifted loam with a moisture content (dry weight basis) of 14 to 17%. Early in these studies this method was found to be very satisfactory for both storage and conditioning of infested stubs. At low temperatures storage can be maintained for many months without harm; at higher temperatures the fibrous structure of the stubs breaks down after about three months, and many larvae mould and die.

In some of the work it was necessary to remove the larvae from their stubs and cocoons and retain them for observation. As naked specimens are an easy prey to desiccation on the one hand and moulds on the other, it was difficult to develop a satisfactory rearing technique. Dryness was the lesser of the two evils, as it merely inhibited development, whereas excessive moisture resulted in a high mortality. A fairly suitable method, and one that was of necessity adopted, was to place the specimens in 1 in. lengths of 1.5 to 2.0 mm. (inside diameter) glass tubing plugged at each end with cotton batting. The small air space was presumably kept fairly moist by evaporation from the specimen, thus producing a reasonably satisfactory microclimate. This technique worked fairly well if the specimen had already begun developing, but not otherwise. Whenever the experiment allowed, the insects were left in the stubs in moist soil as long as possible, usually for 20 days at 25° C.

Samples of stubs, when split open, often yielded specimens in various stages of development. A mere list of the number or percentage of each stage or substage is not only unwieldy but it fails to give a quick means of judging the average extent of development. To partially remedy this situation a numerical rating was given to each developmental category, from which a single value, the developmental index, was derived. The range of development within a sample is not indicated, but the average is given at a glance. Since we are accustomed to thinking in terms of percentage, the index has a base of 100 so that it can be thought of in terms of average percentage of development. The factors were chosen on the basis of the approximate duration of each stage, since development from prepupa to adult at constant favourable conditions is a function of time. Since the time intervals from prepupa to early (uncoloured) pupa, early pupa to late (coloured) pupa, and late pupa to adult are approximately equal, and each is about half as long as that between non-diapause S-larva and prepupa, the following factors are suitable and are used in this work.

S-larva	0
Prepupa	2
Early pupa	3
Late pupa	4
Adult	5

The S-larva, being a non-developing form, naturally receives a zero rating. The percentage of each category present in a sample is multiplied by its factor, and the sum of the products is divided by five, giving a developmental index ranging from 0 to 100. In experimental work where only the developing forms are to be considered the S-larvae may be excluded from the samples and the percentage based on developing forms only (*D.I./x*, where *x* equals developing forms only, rather than *D.I./s*, where *s* equals the total sample). For example, if a sample of 40 specimens contains 20 S-larvae, 10 prepupae, 6 early pupae, 2 late pupae, and 2 adults, the developmental index of the complete sample (*D.I./s*) will be 28, computed as follows:

$$\frac{50(0) + 25(2) + 15(3) + 5(4) + 5(5)}{5}.$$

The inclusion of the S-larvae presupposes that they are postdiapause and will shortly develop into prepupae. If, however, it is known that they are in diapause and will therefore not develop with the remainder of the sample without special conditioning, they should be omitted in the calculations, and the developmental index (*D.I./x*) calculated as follows:

$$\frac{50(2) + 30(3) + 10(4) + 10(5)}{5}.$$

In this case the index is 56. The choice of index therefore depends on whether the S-larvae are in or out of diapause. If this is not known the specimens should be given an opportunity to develop further and the *D.I./x* index used, where *x* equals the number of specimens ultimately showing development, i.e. reaching at least the prepupal stage.

In all of the work included in this paper the prepupa was distinguishable from the S-larva by appearance only. It is of course obvious that after diapause has been eliminated, the change from S-larva to prepupa requires time. Since the process has a starting point, the insect strictly speaking is a prepupa from that time on, rather than from the somewhat later time at which it becomes visually distinguishable as a prepupa. The intervening period is of great practical importance, for, as will be shown later, a post-diapause S-larva can be returned to a state of diapause before the change to prepupa begins, but not after it begins, even though it still has the appearance of an S-larva. Physiologically, the latter is a prepupa; morphologically, it is an S-larva. To avoid confusion it will be termed an S-prepupa.

Experimental Results

1. *Effects of High Temperatures on Postdiapause Development*

In a previous study dealing with the desiccation of *Cephus cinctus* larvae (8), it had become apparent that high temperatures (35°, 40° C.) adversely affected the development of postdiapause larvae, either preventing development entirely when the exposure was made on early postdiapause larvae, or else resulting in abnormalities, coma, and death when more developed forms were used. In a preliminary test 60 isolated naked larvae that were definitely postdiapause were exposed to a temperature of 35° C., half at 50% and half at 80% relative humidity. Observations were made frequently over a period of three weeks, during which no visible change occurred in the 50% relative humidity group and three became prepupal in the 80% relative humidity group. These three were not normal, and it seems probable that they were so close to becoming prepupal at the start of the experiment that development proceeded in spite of adverse conditions that made such development abnormal.

From this it was established only that the temperature and humidity conditions used interfered with normal development of naked postdiapause larvae. In order to remove the moisture factor as a variable and also any possible effects due to removal from the stubs and cocoons, subsequent exposures to high temperatures were made on larvae in their stubs, in damp soil that provided optimum or near-optimum moisture conditions. Three series were exposed to 35° C., each differing in pre-experimental treatment, as follows. In Series *A*, started Jan. 12, 1945, the stubs had been stored in a root cellar during the preceding 113 days; in Series *B*, started Mar. 14, 1945, the stubs had been stored in a root cellar during the preceding 174 days; in Series *C*, started Mar. 24, 1945, the stubs were collected from a wheat field two days earlier. At the end of successive five-day intervals, from 5 to 40 days, stubs were removed and split until 20 specimens were obtained from each series. No development occurred in any sample of any series. Development, therefore, was either prohibited or else the larvae were returned to diapause. The alternatives were tested, in Series *B* and *C*, by removing duplicate samples of stubs from 35° C. at each five-day interval from 5 to 30

days. Instead of being split at once, these samples were incubated at 25° C., still in moist soil, for a further 20 days, at which time the stubs were split and development recorded. Table I lists the percentage that showed visible development after such treatment.

TABLE I

PERCENTAGE OF DEVELOPING SPECIMENS AFTER 5 TO 30 DAYS AT 35° C., FOLLOWED BY 20 DAYS AT 25° C.

No. of days at 35° C.	Series *B*	Series *C*
5	95	100
10	10	60
15	0	20
20	5	5
25	0	0
30	0	0

It is seen that postdiapause larvae that were exposed to 35° C. for a sufficiently long period (25 days or more) were unable to develop after being returned to favourable conditions, or in other words, diapause was reinstated. If the period of exposure was reduced below 25 days, the proportion of developing specimens increased; virtually all specimens developed after only five days at 35° C. Thus a very definite amount of time is necessary to put a larva back into diapause, though this time varies among larvae. Of the two series above, Series *B* was the more advanced. Less time was required to produce diapause in it than in Series *C*, indicating that each larva must reach a certain point or condition before the high temperature produces its effect.

The objection might be raised that 20 days' incubation at 25° C. was insufficient to allow recovery from the high temperature in the event that there were any effects other than the reinstatement of diapause. For this reason Series *C* was duplicated on May 29, 1945, using stubs from the same collection as before, but that had been stored in damp soil at 2° C. during the intervening 68 days. In addition, an extra supply of these stubs was exposed to 35° C. for 25 days. These were then placed at 25° C. and sampled at 10, 15, 20, 25, 30, 40, 50, and 60 days thereafter. The results closely paralleled those of Series *C* in Table I In the extra 25-day samples no development occurred even after incubation at 25° C. for as much as 60 days. The conclusion that the larvae were actually returned to a lasting diapause seems amply justified.

Provided that moisture conditions are suitable, a temperature of 35° C. has no deleterious effects on diapause larvae (8), while in non-diapause larvae, diapause is reinstated. Its effects on prepupae and pupae are quite different, resulting in comatose and abnormal forms. Postdiapause larvae were allowed

to develop in their stubs at 25° C. for various periods, after which they were held at 35° C. for periods of 10 and 20 days. The results are presented in Table II.

TABLE II

EFFECTS OF A TEMPERATURE OF 35° C. ON *Cephus cinctus* AFTER POSTDIAPAUSE DEVELOPMENT HAS STARTED

Days at 25° C.	Days at 35° C.	Percentage				Percentage of developing forms
		S-larvae	Prepupae	Early pupae	Late pupae	
5	10	100	0	0	0	0
5	20	100	0	0	0	0
10	10	40	60	0	0	60
10	20	50	50	0	0	50
15	10	0	80	20	0	100
15	20	7	62	31	0	93
20	10	5	50	5	40	95
20	20	18	29	6	47	82
25	10	0	0	5	95	100
25	20	0	5	0	95	100

Virtually all of the *developing* specimens were comatose or dead, the latter being either moulded or flaccid. It was obvious throughout the course of the experiment that the higher temperature had halted development at whatever point had been reached at 25° C. In other words, development was prohibited at 35° C. There were no apparent ill effects on the non-developing S-larvae but prepupae and pupae lapsed into a coma from which they did not recover. Abnormalities appeared in the prepupae, many of which slowly acquired a semipupal appearance without moulting. First the head darkened and became shaped somewhat like a pupal head; then the thorax gradually darkened, to resemble the form and coloration of the late pupa, but without legs or wings. No further major morphological changes occurred, and the specimens gradually died, sometimes after surviving three or four weeks.

2. Effects of Size on Postdiapause Development

There is considerable variation in size among mature *Cephus cinctus* larvae, attributable mainly to differences in the nutrition of the feeding larvae, which in turn is dependent largely on the growth and vigour of the host plant (2). It became rather noticeable in the course of rearing larvae through to the prepupal, pupal, and adult stages that the larger specimens usually developed sooner. This is not to say that the larger ones developed at a faster rate than the smaller ones, but that they started developing sooner. The speed-up occurs early in the postdiapause period, certainly before the appearance of the prepupa. At present the details are lacking, but a possible explanation of the phenomenon is discussed later.

A sample of 92 specimens, incubated at 25° C. for 20 days in soil of 14% moisture content was divided into groups on the basis of development, weighed, and the moisture and dry matter contents determined by oven-drying to constant weight at 95° to 100° C. These data are listed in Table III.

TABLE III

COMPARATIVE WEIGHTS OF DEVELOPING AND NON-DEVELOPING STAGES IN A SAMPLE OF INCUBATED POSTDIAPAUSE *Cephus cinctus*

——	Number	Average weight, mgm.	Average dry matter, mgm.	Average moisture content, %
S-larvae	15	4.2	1.6	61.5
Prepupae	17	5.0	1.8	63.0
Early pupae	60	8.5	3.2	62.3

There is no significant difference in moisture or dry matter contents, although these differed in absolute amounts. The more advanced forms were definitely larger and heavier than the less advanced.

This point was checked again in the course of another experiment in which each pupa was weighed as soon as it was formed. Table IV lists the number of days required by these specimens to pupate at 25° C.

TABLE IV

RELATION BETWEEN SIZE AND NUMBER OF DAYS TO PUPATION

No. of days to pupation at 25° C.	No. of specimens	Average weight, mgm.
20-21	25	10.1
22	17	8.2
23	14	5.9
24	5	6.7
25	3	5.7
26-28	17	5.4

Again it is evident that in general the larger specimens are more advanced in a random sample of uniformly treated stubs.

3. *Effects of Moderate Temperatures on Diapause in Cephus cinctus*

Not a single case has been recorded of the elimination of diapause at moderate temperatures (20° to 30° C.). Thousands of infested stubs have been stored at room temperature and at constant temperatures within this range, under a variety of moisture conditions, for periods of up to 16 months. Not only is diapause not broken at such temperatures, but there is no indication of any effect whatsoever on it. It is therefore concluded that diapause in *Cephus cinctus* is not only obligatory but is also maintained at moderate and high temperatures.

4. *Effects of Low Temperatures on Diapause in Cephus cinctus*

The diapause present in every *Cephus cinctus* larva in the fall is broken by spring. It was at first largely taken for granted that low temperatures were responsible for the change. If postdiapause material was needed for winter experimental work, fall-collected stubs were placed on or near the coils of a refrigerator or in a root cellar for the greater part of the winter. This procedure was quite successful when sufficient conditioning was allowed. No precise information was at hand, however, to indicate what temperature conditions were most suitable, or whether the time factor could be reduced. In order to get such information, attempts were made in the fall of 1944 to break diapause under controlled conditions.

The first exposures were made on Sept. 8, 1944, using infested stubs collected the previous day. The temperature of the refrigerator varied between 2° and 5° C. Stubs and isolated naked larvae were exposed, with and without contact moisture, in groups of 50 and 25, respectively, for 5, 10, 15, and 20 days. A control sample of stubs was held at 30° C. and 70% relative humidity, and the experimentals were also held at these conditions following the low temperature treatment. All exposures were unsuccessful in breaking diapause, as not a single larva became prepupal. Later work indicates that the exposure periods were insufficient. In addition, however, the postexperimental treatment was too hot and too dry for satisfactory postdiapause development. Nevertheless, if diapause had been broken, the occasional individual would have developed to at least the prepupal stage at 30° C. and 70% relative humidity. Since this did not occur, the conclusion that exposures of 2° to 5° C. for periods up to 20 days are insufficient to break diapause is substantially correct.

On Sept. 12 and 15, 1944, further exposures were made at temperatures of −15° and −20° C., using larvae from stubs collected on Sept. 9. Larvae were removed from their stubs and exposed in clean honeycomb, after which they were held at 30° C. and 70% relative humidity for observation. At −15° C. larvae were exposed for five hours, one day, three days, and seven days; at −20° C. for three hours, five hours, one day, two days, and three days. There was no development following any of these treatments, and the remarks made above concerning the previous experiment also apply here.

No further experiments were carried on at this time, as no more diapause larvae were available. In the meantime, however, the conditions for re-establishment of diapause were being worked out, and in the spring a collection of stubs was made and exposed to 35° C. for 30 days to provide diapause larvae for further experimentation. A control sample proved to be in diapause; the remainder were held at 5° and 10° C. for periods of 20, 30, 45, and 60 days, followed by an incubation period of 20 days at 28° C. All stubs were kept in moist soil. No visible development occurred in any sample, and it was concluded that the treatments were either ineffective or insufficient. In comparison with a later experiment, in which, however, the diapause was natural and not artificially induced, it appears that the 60 day

exposure to 10° C. should have broken diapause in some individuals at least. Since this was not the case, it may indicate a quantitative difference between the fall and spring types of diapause.

Half of the stubs conditioned at 5° and 10° C. for 20 and 30 days were not split open after their incubation period at 28° C., but were reconditioned for an additional 20 days, while the two 45 day samples were given an additional 30 days. This was followed by a second 20 day incubation period, this time at 25° C. Again there was no development.

The only successful breaking of diapause during the 1944-45 season occurred in a small sample exposed to 10° C. for 80 days. These larvae were definitely in artificial diapause beforehand. After an incubation period of 20 days at 25° C. 17 out of 19 specimens showed visible development, 15 being early pupae and two prepupae. The developmental index was 52.

Tests made during the 1945-46 season were much more successful. On Aug. 29, 1945, freshly collected stubs containing larvae in natural fall diapause were placed in soil of 17% moisture content at 5° and 10° C. and in a root cellar. After periods of 40, 50, 60, 70, 80, 90, and 100 days, samples were incubated for 20 days at 25° C. Stubs were then split until 20 living specimens were obtained. Dead larvae were disregarded, as they were either dead before the experiment or else died for reasons not pertinent to the experiment. There was no mortality among later stages (in the stubs), and no parasitism. Following removal from the stubs on the 20th day of incubation, the specimens were placed in 1 in. lengths of 1.5 to 2.0 mm. glass tubing, at 25° C., for further observation. As explained above, this method proved fairly satisfactory for individuals that had already begun postdiapause development, but not for those that had not. The best comparison among samples is therefore obtained on the 20th day at 25° C. when the stubs were split. However, comparisons can also be made of subsequent development, as the microclimatic conditions within the tubes were presumably similar. Tables V, VI, and VII list the developmental data; in the case of the 5° C. series, the time was extended to include samples at 140 and 150 days.

Comparing the three tables with each other it is apparent that diapause was eliminated much sooner at 10° C. than at 5° C., and somewhat sooner at 10° C. than in the root cellar. This comparison, it should be noted, is on the basis of time only; larvae held at 5° C. came out of diapause just as well as those at 10° C., but the conditioning period required was considerably longer. It appears probable that the time required at temperatures below 5° C. is still longer, which would explain the lack of success in the experiments previously discussed, where temperatures of 2° to 5°, −15°, and −20° C. were used. The optimum temperature, or the one at which diapause is broken most rapidly, must lie not far from 10° C. Not far above 10° C. lies the developmental range, in which diapause cannot be broken by temperature.

The effects of low temperature on diapause are best seen by the data in Table VI. A period of 40 days at 10° C. was insufficient to break the diapause

TABLE V

PERCENTAGE OF INDIVIDUALS DEVELOPING, AND DEVELOPMENTAL INDICES (*D.I./x*, WHERE *x* EQUALS THE NUMBER OF SPECIMENS ULTIMATELY DEVELOPING) AFTER CONDITIONING DIAPAUSE *Cephus cinctus* LARVAE AT 5° C. FOR 40 TO 150 DAYS

Days at 25° C.	Days at 5° C.								
	40-80[1]	90		100		140		150	
		A[2]	*B*[3]	*A*	*B*	*A*	*B*	*A*	*B*
20		10	40	20	50	95	54	100	65
21		—	—	—	—	95	56	100	70
22		10	40	20	55	95	60	—	—
23		—	—	—	—	—	—	100	84
24		—	—	20	55	95	68	100	90
25		10	50	—	—	—	—	—	—
26		—	—	20	60	95	78	100	94
27		10	50	—	—	95	81	—	—
28		—	—	—	—	95	85	100	97
29		—	—	20	75	95	88	—	—
30		10	50	—	—	—	—	100	99
31		—	—	20	85	95	93	100	100
32		10	70	—	—	—	—		
33		—	—	20	90	95	97		
34		10	90	—	—	95	99		
35		—	—	—	—	95	100		
36		10	100	20	95				
37				—	—				
38				20	100				

[1] *No development.*
[2] *A = percentage developing.*
[3] *B = D.I./x.*

of any larva. It was broken in 38% after 50 days at 10° C.; 70% after 60 days; 85% after 70 days; 80% after 80 days; 95% after 90 and 100 days. There are minor irregularities, but in general the increase is proportional to the conditioning period. An exposure of 110 days at 10° C. would no doubt have been effective in all cases. It is apparent, however, that the time required to break diapause varies considerably among individuals. Some required fewer than 50 days at 10° C.; a few more than 90 days; and the majority from 60 to 80 days. Thus it is not correct to speak of diapause being gradually broken during the conditioning period if reference is made to individuals; in a group, however, this is correct.

The progress of diapause elimination under outside conditions may be followed in Table VIII, which lists the developmental indices and the percentage of developing individuals in samples of infested stubs brought into the laboratory from the field at weekly intervals from Oct. 5, 1945, to March 1, 1946. Each sample was incubated at 25° C. for 20 days, in soil of 17% moisture content.

The progressive elimination of diapause shown in Table VIII is essentially the same as that in Tables V, VI, and VII, but it is more complete, and in

TABLE VI

PERCENTAGE OF INDIVIDUALS DEVELOPING, AND DEVELOPMENTAL INDICES (*D.I./x*, WHERE *x* EQUALS THE NUMBER OF SPECIMENS ULTIMATELY DEVELOPING) AFTER CONDITIONING DIAPAUSE LARVAE OF *Cephus cinctus* AT 10° C. FOR 40 TO 100 DAYS

Days at 25° C.	Days at 10° C.											
	40[1]	50		60		70		80		90		100	
		A[2]	*B*[3]	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*
20		0	0	10	6	50	25	75	39	85	47	95	56
21		—	—	—	—	—	—	—	—	—	—	—	—
22		0	0	—	—	—	—	—	—	90	54	95	57
23		—	—	—	—	—	—	80	53	—	—	—	—
24		0	0	—	—	60	38	—	—	—	—	95	65
25		—	—	25	14	—	—	80	56	95	63	—	—
26		0	0	—	—	—	—	—	—	—	—	95	74
27		—	—	—	—	70	45	—	—	95	74	—	—
28		8	8	—	—	—	—	80	64	—	—	—	—
29		—	—	—	—	—	—	—	—	—	—	95	81
30		15	16	70	57	—	—	80	74	95	84	—	—
31		—	—	—	—	85	63	—	—	—	—	95	88
32		23	28	—	—	—	—	80	87	95	89	—	—
33		—	—	—	—	85	70	—	—	—	—	95	94
34		23	32	70	71	—	—	—	—	95	97	—	—
35		—	—	—	—	85	78	80	94	—	—	—	—
36		31	48	—	—	—	—	—	—	95	97	95	97
37		—	—	70	86	—	—	80	95	—	—	—	—
38		31	56	—	—	85	79	—	—	—	—	95	100
39		—	—	—	—	—	—	—	—	95	99		
40		31	64	—	—	85	85	80	98	—	—		
41		—	—	70	96	—	—	—	—	95	99		
42		38	80	—	—	85	89	80	100	—	—		
43		—	—	70	100	—	—			95	100		
44		38	80			—	—						
45		—	—			85	96						
46		—	—			—	—						
47		38	84			85	99						
48		—	—			—	—						
49		—	—			—	—						
50		—	—			85	100						
51		38	88										
52		—	—										
53		38	92										
54		—	—										
55		38	92										
56		—	—										
57		—	—										
58		38	96										
59		—	—										
60		38	100										

[1] *No development.*
[2] *A = percentage developing.*
[3] *B = D.I./x.*

addition it gives some idea of what actually occurs during the fall and winter under natural conditions. For instance, 10% of the larvae were postdiapause by Oct. 19, 1945, and 25% by Nov. 2. The intervening sample, with 65% postdiapause larvae on Oct. 26, is greatly out of line with the other samples.

The only explanation offered is that of mere chance, as a result of the small size of the sample (20 specimens). Diapause was broken in more and more specimens throughout the winter, and was complete by the end of January, 1946.

TABLE VII

PERCENTAGE OF INDIVIDUALS DEVELOPING, AND DEVELOPMENTAL INDICES (*D.I./x*, WHERE *x* EQUALS THE NUMBER OF SPECIMENS ULTIMATELY DEVELOPING) AFTER CONDITIONING DIAPAUSE LARVAE OF *Cephus cinctus* IN A ROOT CELLAR FOR 40 TO 100 DAYS

Days at 25° C.	Days in root cellar											
	40[1]	50		60		70		80		90		100	
		A[2]	*B*[3]	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*
20		0	0	10	6	5	4	60	31	65	36	55	41
21		—	—	—	—	—	—	—	—	—	—	—	—
22		—	—	—	—	—	—	—	—	75	42	55	43
23		—	—	—	—	—	—	70	44	—	—	—	—
24		—	—	—	—	5	4	—	—	—	—	70	54
25		—	—	20	18	—	—	75	53	80	52	—	—
26		—	—	—	—	—	—	—	—	—	—	70	59
27		—	—	—	—	5	4	—	—	80	61	—	—
28		—	—	—	—	—	—	75	58	—	—	—	—
29		—	—	—	—	—	—	—	—	—	—	70	70
30		—	—	50	40	—	—	75	59	80	66	—	—
31		—	—	—	—	35	33	—	—	—	—	70	77
32		—	—	—	—	—	—	75	69	80	73	—	—
33		—	—	—	—	35	38	—	—	—	—	70	86
34		—	—	55	54	—	—	—	—	85	85	—	—
35		—	—	—	—	35	45	80	82	—	—	—	—
36		—	—	—	—	—	—	—	—	85	89	70	91
37		—	—	60	60	—	—	80	89	—	—	—	—
38		—	—	—	—	45	53	—	—	—	—	70	96
39		—	—	—	—	—	—	—	—	85	95	—	—
40		—	—	—	—	45	62	80	91	—	—	70	100
41		—	—	65	74	—	—	—	—	85	98		
42		—	—	—	—	45	73	80	93	—	—		
43		—	—	65	85	—	—	—	—	85	99		
44		5	40	—	—	—	—	80	95	—	—		
45		—	—	65	88	45	84	—	—	—	—		
46		—	—	—	—	—	—	80	99	85	100		
47		10	70	—	—	45	87	—	—				
48		—	—	65	94	—	—	—	—				
49		—	—	—	—	—	—	80	100				
50		—	—	65	94	45	89						
51		10	80	—	—	—	—						
52		—	—	65	95	45	93						
53		10	80	—	—	—	—						
54		—	—	—	—	45	98						
55		10	80	65	97	—	—						
56		—	—	—	—	45	100						
57		—	—	65	97								
58		10	90	—	—								
59		—	—	—	—								
60		10	90	65	100								
61		—	—										
62		10	100										

[1] *No development.*
[2] *A = percentage developing.*
[3] *B = D.I./x.*

TABLE VIII

TABLE VIII

PERCENTAGE OF DEVELOPING INDIVIDUALS AND DEVELOPMENTAL INDICES (*D.I./x*, WHERE *x* EQUALS THE NUMBER OF SPECIMENS ULTIMATELY DEVELOPING) FOLLOWING NATURAL OUTDOOR CONDITIONING. SAMPLES TAKEN WEEKLY FROM OCT. 5, 1945 TO MAR. 1, 1946

Days at 25° C.	Outdoors until:																																	
	Oct. 5[1]		Oct. 17[1]		Oct. 19		Oct. 26		Nov. 2		Nov. 9		Nov. 16		Nov. 23		Nov. 30		Dec. 7		Dec. 14		Dec. 21		Dec. 28		Jan. 4		Jan. 11[4]		Jan. 18[4]		Jan. 25		Feb. 1		Feb. 8		Feb. 15		Feb. 22		Mar. 1	
					A[2]	B[3]	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B					A	B	A	B	A	B	A	B	A	B	A	B
20					0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	40	25	55	32	60	28	70	35					90	47	90	46	100	47	95	58	100	58	100	63
21					—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	80	40					95	50	90	48	100	52	95	59	—	—	—	—
22					—	—	—	—	—	—	—	—	0	0	0	0	0	0	0	0	60	38	60	36	70	38	80	45					95	50	90	50	100	58	95	63	100	65	100	76
23					—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	85	52					95	54	90	53	—	—	—	—	—	—	—	—
24					—	—	—	—	—	—	0	0	0	0	0	0	33	16	23	15	65	46	70	49	75	44	85	53					95	55	95	58	100	61	95	66	100	75	100	93
25					—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—					95	55	95	58	—	—	—	—	—	—	100	96
26					—	—	—	—	—	—	—	—	0	0	0	0	—	—	41	25	65	51	75	52	75	48	—	—					95	57	95	63	100	67	95	75	100	88	—	—
27			0	0	—	—	—	—	—	—	—	—	—	—	—	—	61	29	—	—	—	—	—	—	—	—	85	59					100	61	95	67	100	69	95	85	—	—	100	100
28					—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	85	55	85	63					100	66	95	74	100	80	—	—	—	—		
29					—	—	—	—	—	—	0	0	10	8	0	0	78	44	59	40	65	60	75	61	85	58	85	67					100	72	95	79	100	82	95	94	100	98		
30					—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	85	61	85	75					100	76	—	—	—	—	—	—	—	—		
31					—	—	—	—	0	0	5	7	10	8	5	5	78	47	59	45	65	66	75	69	85	65	85	76					100	84	95	91	100	94	95	99	100	100		
32					—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	85	82					100	90	—	—	—	—	—	—	—	—		
33					—	—	—	—	—	—	5	7	40	36	—	—	83	55	65	53	65	75	75	76	—	—	85	89					100	92	95	96	100	99	95	100				
34					—	—	—	—	—	—	—	—	—	—	25	25	—	—	—	—	—	—	—	—	85	80	85	94					100	95	95	99	100	100						
35					—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	75	87	85	84	85	95					100	97	95	100								
36					—	—	—	—	10	16	5	10	40	40	25	27	83	60	65	60	65	85	75	89	85	87	85	96					100	97										
37					—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	75	93	85	93	85	98					—	—										
38					65	49	20	40	5	10	40	48	25	35	83	68	65	67	65	94	75	96	85	95	85	100					100	98										
39					—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	85	97							—	—										
40					—	—	—	—	25	48	15	23	40	52	30	40	83	75	65	74	63	97	75	100	85	98					100	99										
41					—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	85	100							—	—										
42					—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	65	100											100	100										
43					—	—	65	62	25	56	25	43	40	52	35	50	83	88	65	84																						
44					—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—																						
45					10	40	65	63	25	60	25	43	40	60	35	60	83	93	65	89																						
46					—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—																						
47					—	—	65	72	25	68	—	—	50	76	40	67	83	96	65	96																						
48					—	—	—	—	—	—	30	57	—	—	—	—	—	—	—	—																						
49					—	—	—	—	—	—	—	—	—	—	—	—	—	—	65	98																						
50					10	60	65	88	25	80	30	60	50	84	40	80	83	100	—	—																						
51					—	—	—	—	—	—	—	—	—	—	—	—	—	—	65	100																						
52					10	60	69	91	25	88	30	60	50	96	40	82																										
53					—	—	—	—	—	—	—	—	—	—	—	—																										
54					10	60	65	97	—	—	30	67	50	96	40	90																										
55					—	—	—	—	25	100	—	—	—	—	—	—																										
56					—	—	—	—	—	—	—	—	—	—	—	—																										
57					10	70	65	97			30	77	50	100	40	92																										
58					—	—	—	—			—	—			—	—																										
59					10	80	65	97			30	80			40	97																										
60					—	—	—	—			—	—			—	—																										
61					10	80	—	—			30	83			40	100																										
62					—	—	65	100			—	—																														
63					—	—					—	—																												
64					10	90					30	93																												
65					—	—					—	—																												
66					10	90					30	93																												
67					—	—					—	—																												
68					—	—					30	100																												
69					10	100																																		

[1] *No development.* [2] *A = percentage developing.* [3] *B = D.I./x.* [4] *No samples.*

Apart from the progressive elimination of diapause, Tables V to VIII have one other feature in common. As the conditioning period lengthened, not only did the percentage of developing specimens increase, but the time required for these specimens to begin developing steadily decreased. In other words, the longer the exposure at the conditioning low temperature, the *sooner* development starts at the higher temperature, within limits. This can be traced most accurately by comparing the developmental indices (*D.I./x*, where *x* equals the number of specimens ultimately developing) on the 20th day of incubation, the day on which the stubs were split. These data are brought together in Tables IX and X. In each case the developmental index steadily increased as the low-temperature conditioning period lengthened. The limit to this process, for there must be one, has not as yet been determined.

TABLE IX

DEVELOPMENTAL INDICES AND PERCENTAGE DEVELOPING ON THE 20TH DAY OF INCUBATION ONLY. DATA FROM TABLES V, VI, AND VII

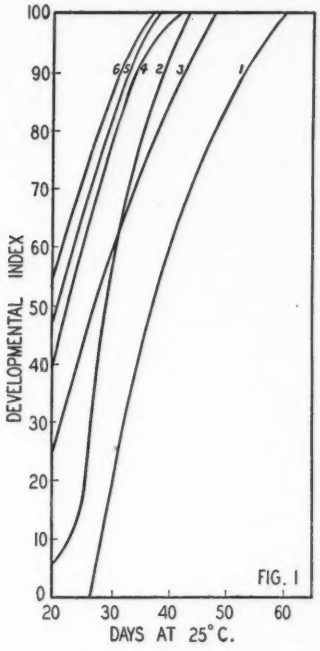
Number of days	5° C.		10°C.		Root cellar	
	% Developing	*D.I./x*	% Developing	*D.I./x*	% Developing	*D.I./x*
40	0	0	0	0	0	0
50	0	0	0	0	0	0
60	0	0	10	6	10	6
70	0	0	50	25	5	4
80	0	0	75	39	60	31
90	10	40	85	47	65	36
100	20	50	95	56	55	41
140	95	54	—	—	—	—
150	100	65	—	—	—	—

TABLE X

DEVELOPMENTAL INDICES AND PERCENTAGE DEVELOPING ON THE 20TH DAY OF INCUBATION ONLY. DATA FROM TABLE VIII

Date	% Developing	*D.I./x*	Date	% Developing	*D.I./x*
Oct. 5, 1945	0	0	Dec. 21, 1945	55	32
12	0	0	28	60	28
19	0	0	Jan. 4, 1946	70	35
26	0	0	11	—	—
Nov. 2	0	0	18	—	—
9	0	0	25	90	47
16	0	0	Feb. 1	90	46
23	0	0	8	100	47
30	0	0	15	95	58
Dec. 7	0	0	22	100	58
14	40	25	Mar. 1	100	63

For convenience, the data in Table VI and most of those in Table VIII are presented graphically in Figs. 1 and 2, respectively. The developmental curves have been smoothed somewhat and in Fig. 2 every second curve of the earlier samples has been omitted to avoid confusion caused by overlapping.



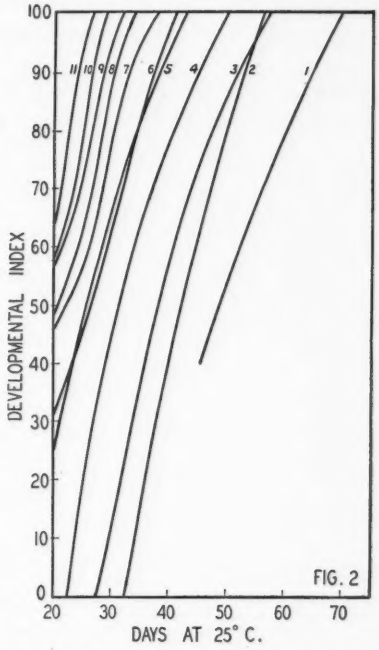


FIG. 1. *Development of Cephus cinctus at 25° C. following various conditioning periods at 10° C.*

1. 50 days at 10° C.
2. 60 " " "
3. 70 " " "
4. 80 days at 10° C.
5. 90 " " "
6. 100 " " "

No development after 40 days at 10° C.

FIG. 2. *Development of Cephus cinctus at 25° C. after being brought indoors from the field at various dates.*

1. Oct. 19, 1945.
2. Nov. 2, "
3. Nov. 16, "
4. Nov. 30, "
5. Dec. 14, "
6. Dec. 28, "
7. Jan. 25, 1946.
8. Feb. 8, "
9. Feb. 15, "
10. Feb. 22, "
11. Mar. 1, "

No development in samples brought indoors previous to Oct. 19, 1945.

Both figures demonstrate quite clearly the fact that as the period of low-temperature conditioning is increased, the time required to begin postdiapause development is decreased. It is also clearly apparent from the similar slope of the curves that the *rate* of development, after it has once begun, is rather constant. Thus there was no acceleration of development itself; instead there

was a shortening of the postdiapause but predevelopmental period. A discussion of this is given in the next section.

Discussion

There is a critical period in the life cycle of *Cephus cinctus* when diapause can recur after having once been broken. Under natural conditions this period occurs in the spring, following the breaking of the obligatory fall diapause, but preceding active spring development (i.e. before the appearance of the prepupa). For this reason it is called spring diapause to distinguish it from the obligatory fall diapause. Under laboratory conditions spring diapause is readily produced at high temperatures (35° C.). There is good evidence that insufficient moisture at this critical period also reinduces diapause, but this subject is reserved for a later paper.

Although the matter has not been especially investigated, there seems to be no evidence of any qualitative difference between fall and spring diapause. There is slight evidence of a quantitative difference, in that the low temperature conditioning periods required to break the two diapauses may differ, but this is not at all certain.

A temperature of 35° C. has no apparent ill effect in itself on larvae in diapause. It has definite ill effects on developing forms such as prepupae and pupae, in which it produces coma, abnormalities, and death. It reinstates diapause only during the intervening stage, that of the semidiapause S-larva. In all of the present experimental work this stage has been delimited only by indirect means. By conditioning larvae for an ample period at low temperatures the breaking of diapause is effected, and the larvae have been assumed to be postdiapause from the instant they are transferred from the low to the incubation temperature. The S-larval stage has been considered as ended only when the prepupa becomes visually recognizable. This practice is obviously not precise, as the morphological changes distinguishing prepupa from S-larva require some time. Just how much time is actually required is unknown, but since this is a critical period in the life cycle it is of considerable practical importance. The course of the respiratory rate during this crucial period has not been followed as yet, but plans have been made for such a study. It is likely to give a more clear-cut separation of the various stages, especially if diapause in *Cephus cinctus* proves to be similar to that in eggs of *Melanoplus differentialis*. Bodine (1) found that during diapause the respiratory rate of *M. differentialis* eggs is depressed. When postdiapause development begins, the rate rises appreciably, and thus can be used as a test for the presence or absence of diapause. If *Cephus cinctus* reacts similarly, the respiratory rate would rise, at favourable temperatures, as soon as diapause is *completely* eliminated. This point would then mark the true beginning of the prepupal stage, and in addition it would mark the end of the insect's ability to return to diapause.

We have seen, in this connection, that the insect does not in most instances begin to develop as soon as it is brought to the incubation temperature. It

has been shown in Tables V to VIII and Figs. 1 and 2 that as the exposure to the conditioning low temperature is increased, the time until development starts, when measured by the visible change to the prepupa, is decreased. Since some individuals may become prepupal as much as 20 days (at 25° C.) earlier than others, it is apparent that all do not begin to develop at the same time. The difference must occur in the predevelopmental stage here called the "semidiapause S-larva".

The assumption has been made that if the exposure to a low temperature is sufficient to allow postdiapause development at the incubation temperature, diapause is 'broken'. Thus all individuals listed in Tables V to VIII that developed (i.e. reached the prepupal stage) would be 'out of diapause', regardless of the time required for them to start to develop, or reach the prepupal stage. In terms of Bodine's (1) theory of diapause, their *x* factor was eliminated or reduced below the level of effectiveness. Yet it has been shown above that after the *x* factor is inoperative the beginning of postdiapause development must wait for something else—presumably another physiological reaction. Following Bodine's analogy, we can postulate a *y* factor, which remains to be eliminated after *x* has gone. But whereas *x* is eliminated only at low temperatures, *y* is eliminated at both low and high temperatures. Thus it may be correct to say that diapause is 'broken' after the *x* factor has been made inoperative, but it is not 'eliminated' until after the *y* factor has also been made inoperative.

To take a definite example from Table VI, in the sample of larvae conditioned at 10° C. for 50 days the most tardy individual became prepupal on the 41st or 42nd day of incubation at 25° C. In the sample conditioned at 10° C. for 90 days, the most tardy individual became prepupal on the 23rd, 24th, or 25th day. In the additional 40 days at 10° C. the *y* factor was reduced to the same extent as in 16 to 19 days at 25° C. The reduction in time in each case represents a reduction in the *y* factor. Even in the latter sample the *y* factor was far from eliminated after 90 days at 10° C. In Table VIII there is evidence that the *y* factor was not quite eliminated by Mar. 1, 1946. The time at 25° C. required for all ultimately developing specimens to start developing was gradually being reduced. Just where the minimum lay was not determined because of difficulties in the rearing technique that occurred when the larvae had to be reared apart from their protective stubs and cocoons. This point, however, will be investigated later by the use of a respirometer.

A question of terminology naturally arises over the use of the term 'postdiapause'. As soon as the *x* factor has been eliminated, diapause is broken (though not eliminated, for the *y* factor remains) and the insect still has the appearance of an S-larva. It has been stated above in this discussion that it is probable that the beginning of increased respiratory rate, the beginning of the prepupal stage, and the end of the ability to return to diapause all fall at the same time. Now, "the end of the ability to return to diapause" may be changed to "the elimination of the *y* factor" with the understanding that diapause cannot be reinstated when both *x* and *y* factors are gone. The

normal course of events in *Cephus cinctus* would then be as follows. Beginning with an S-larva in diapause, the *x* factor is dissipated at low temperatures. Following this the *y* factor is dissipated at either low or moderately high temperatures. As soon as the *y* factor is completely gone, diapause is over, postdiapause development begins, and the insect is then a prepupa, though it will continue to look like an S-larva for a short time. No ecdysis marks either this or the previous change. Until these various stages or phases are more exactly delimited by means of physiological tests we have a curious mixture of morphological and physiological steps, which may be summarized as follows:

	Morphological form	Physiological form
1. Diapause S-larva (*x* and *y* factors operative)	Larva (non-developing)	Larva (non-developing)
2. Semidiapause S-larva (*y* factor, only, operative)		
3. Postdiapause S-larva = S-prepupa	Prepupa (developing)	Prepupa (developing)
4. Prepupa		

Although, as stated, there is a lack of exact delimitation of the above stages, there is no doubt about their existence. Nor are they of merely theoretical interest. The semidiapause S-larva is readily returned to full diapause and is thus of considerable practical importance. It is of interest to note in this regard that the species has a double protection against postdiapause development in the fall. A small percentage of the larvae had their diapause broken (*x* factor only) on Oct. 19, 1945, but it required between 28 and 45 days at 25° C. for them to start development (Table VIII). In the cooler temperatures of their natural environment, even allowing for periods of comparative warmth, it would be impossible for them to start development before winter. By the time that temperatures are high enough in the spring to permit development, the *y* factor will have been entirely or nearly eliminated, thus wiping out individual differences in *x*-factor elimination.

If it is thought of as a reaction similar to Bodine's *x* factor, the *y* factor is reduced at a faster rate at 25° C. than at 10° C. or 5° C. Although the full range was not specifically investigated, it seems probable that the rate of elimination increases with temperature within limits bounded by the undercooling point on the one hand and a temperature of about 30° C. on the other. (Reinstatement of diapause starts at a temperature of about 30° C. in many cases. Some *y* factor still remains in such specimens.) The *x* factor was eliminated faster at 10° C. than at 5° C., and presumably faster at 5° C. than at lower temperatures. Again, the rate of elimination increased with temperature within limits, this time by the undercooling point on the one hand and a temperature not much above 10° C. on the other. The low upper limit in the case of the *x* factor reaction does not seem to be readily explainable with only the facts already known. If a conjecture is permissible, it would seem that the *x* factor reaction is in some way interfered with at developmental

temperatures. The threshold of development, or at least the threshold of certain developmental processes, lies not far above 10° C., and this is approximately where the upper limit of the *x* factor reaction lies.

The terms "*x* factor" and "*y* factor" are used here more for convenience than anything else. Their nature is at present quite vague and uncertain even though there are a few things we can definitely say of them. We know, for instance, the order of their occurrence or elimination and their reactions to a rather wide range of temperatures. We also know that they are reversible, because a return to full diapause is readily accomplished under certain conditions. This latter fact rules out the possibility of morphological changes being associated with the process of diapause elimination, since morphological changes are not reversible. It seems very likely that the process is chemical, though whether enzymatic, hormone-controlled, or otherwise is at present uncertain.

It has been shown that earliness of postdiapause development in *Cephus cinctus* is related to size. Large larvae generally reach the prepupal and pupal stages well in advance of similarly conditioned small larvae. The difference does not lie in the rate of postdiapause development, but in the time prior to the beginning of this development. Again, it is the semidiapause S-larval stage that varies, and we are faced with the probability that in large larvae the *y* factor is eliminated sooner (possibly also faster) than in small larvae. No attempt is made here to explain why this is so, but the fact that it is so accounts for the rather wide range of development within samples. This variability was the cause of much concern during the earlier part of this experimental work. It was originally planned to set up standard rearing conditions as a basis for comparison, with the expectation that postdiapause development would be rather uniform. While development following the visibly-recognizable prepupal state was reasonably uniform (Figs. 1, 2), the earlier period was far from it. This led to a recognition of the relation of size to *y*-factor elimination, and to the abandonment of the attempt to set up standard conditions, since the stub prevents selection of samples uniform as to size.

Acknowledgments

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THE PROVENTRICULUS OF THE LARVA OF THE CADDIS-FLY, *MACRONEMA ZEBRATUM* HAGEN (TRICHOPTERA: HYDROPSYCHIDAE)[1]

By W. W. Judd[2]

Abstract

The structure of the proventriculus of the larva of *Macronema zebratum* Hagen is described. The form of the organ is similar to that of some other larvae of the Hydropsychidae. It probably functions as a masticator or compressor of food.

Introduction

The structure of the digestive tract of caddis-fly larvae has been studied by a few authors who note a variety of patterns in the arrangement of folds on the sclerotized lining of the proventriculus. Lloyd (4) reports that the later instars of the larvae of the Hydropsychidae are carnivorous and Glasgow (3) reports that the proventriculus of *Hydropsyche colonica* is characterized by 34 to 38 strong, sclerotized teeth provided with small denticles and by great development of the circular muscles. Lloyd (4) and Gätjen (2) state that many of the larvae of the Phryganeidae are herbivorous and that the proventriculus of *Phryganea interrupta* Say in this family (1) is large and has a heavy musculature. In *Anabolia laevis* Zett. Russ (5) shows that the sclerotized lining of the proventriculus is thin and the musculature weak except where the proventriculus joins the oesophageal valve.

Materials

Larvae of *Macronema zebratum* Hagen were collected from the lower surface of stones in shallow water of the Ottawa River at Ottawa, Ont., Sept. 2, 1939. They were identified by Dr. T. H. Frison, Chief of the Illinois Natural History Survey, Urbana, Ill.

The digestive tracts were removed from specimens and pinned to a layer of wax in a Syracuse watch glass and then covered with water. The proventriculus was cut longitudinally with fine scissors. A section including the proventriculus was cut from the digestive tract and was placed for 12 hr. in a solution of potassium hydroxide, which dissolved the muscles and other soft tissues. The sclerotized lining of the organ, divested of muscles, was passed through several changes of water, alcohols, and xylol and then mounted in Canada balsam.

Serial transverse sections (10μ) were made of a number of digestive tracts and were stained with haematoxylin and eosin.

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Part of a thesis presented in accordance with the requirements for the degree of Master of Arts of the University of Western Ontario, London, Ont.

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Description

The proventriculus of the larva of *Macronema zebratum* Hagen is 2 mm. long and lies in the mesothorax or metathorax. It consists of two regions separated by a constriction. The anterior region is 0.75 mm. broad anteriorly and tapers posteriorly toward the constriction. The posterior region is cylindrical and 0.15 mm. broad.

The outer layer of the anterior region consists of heavy circular muscle 0.1 mm. thick (Figs. 3, 4–CM). Next to the circular muscle is the layer of epidermal cells (Fig. 3–EP). Between the epidermal layer and the lumen of the proventriculus is the sclerotized intima. This is organized in the form of 18 longitudinal teeth running the length of the anterior region (Figs. 1, 2, 3, 4–LT). These teeth are flattened laterally (Fig. 3) and their greatest height is attained at points about three-quarters of the length from their anterior ends (Fig. 2). Where their peaks converge they almost block the lumen of the proventriculus (Fig. 4).

In the posterior region of the proventriculus the circular muscle is about 0.05 mm. thick (Figs. 5, 6, 7–CM). Corresponding with the 18 teeth of the anterior region there are in the posterior region 18 longitudinal folds (Figs. 2, 5–LF) that extend posteriorly about one-quarter of the length of the posterior region. They are beset with a heavy coat of strong hairs that converge into the lumen (Figs. 1, 2, 5–CS). Posterior to the longitudinal folds, and bulging downward from the dorsal wall of the proventriculus, is a sclerotized pad (Figs. 1, 6–P) beset with small spines and almost filling the lumen. At each side of the pad there is a longitudinal ridge (Figs. 1, 6–LR). Posteriorly the pad and ridges become laterally flattened and in transverse section appear as three processes projecting downward across the lumen (Fig. 7–P, LR). Small longitudinal folds appear along those parts of the sides of the lumen not occupied by the pad and longitudinal ridges.

The foregoing description of the structure of the proventriculus of the larva of *M. zebratum* indicates that this organ is similar to that of other members of the Hydropsychidae. Branch (1) shows that in one species of *Hydropsychodes* the proventriculus has 36 heavy 'stomachic' teeth; and Glasgow shows that in one species of *Hydropsyche* there are from 34 to 38 strong teeth. It is probable that this organ functions as a masticator or

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FIGS. 1–7. *Figures of the proventriculus of Macronema zebratum showing the flattened sclerotized lining and transverse sections at several levels.*

CM: circular muscle; CS: sclerotized hairs; EP: epidermal cells; LF: longitudinal fold; LR: longitudinal ridge; LT: longitudinal tooth; P: sclerotized pad.

FIG. 1. *Part of the sclerotized lining of the proventriculus showing four longitudinal teeth.*

FIG. 2. *Lateral view of longitudinal tooth.*

FIG. 3. *Transverse section through anterior ends of teeth.*

FIG. 4. *Transverse section through peaks of teeth.*

FIG. 5. *Transverse section through sclerotized folds.*

FIG. 6. *Transverse section through anterior end of sclerotized pad.*

FIG. 7. *Transverse section through posterior end of sclerotized pad.*

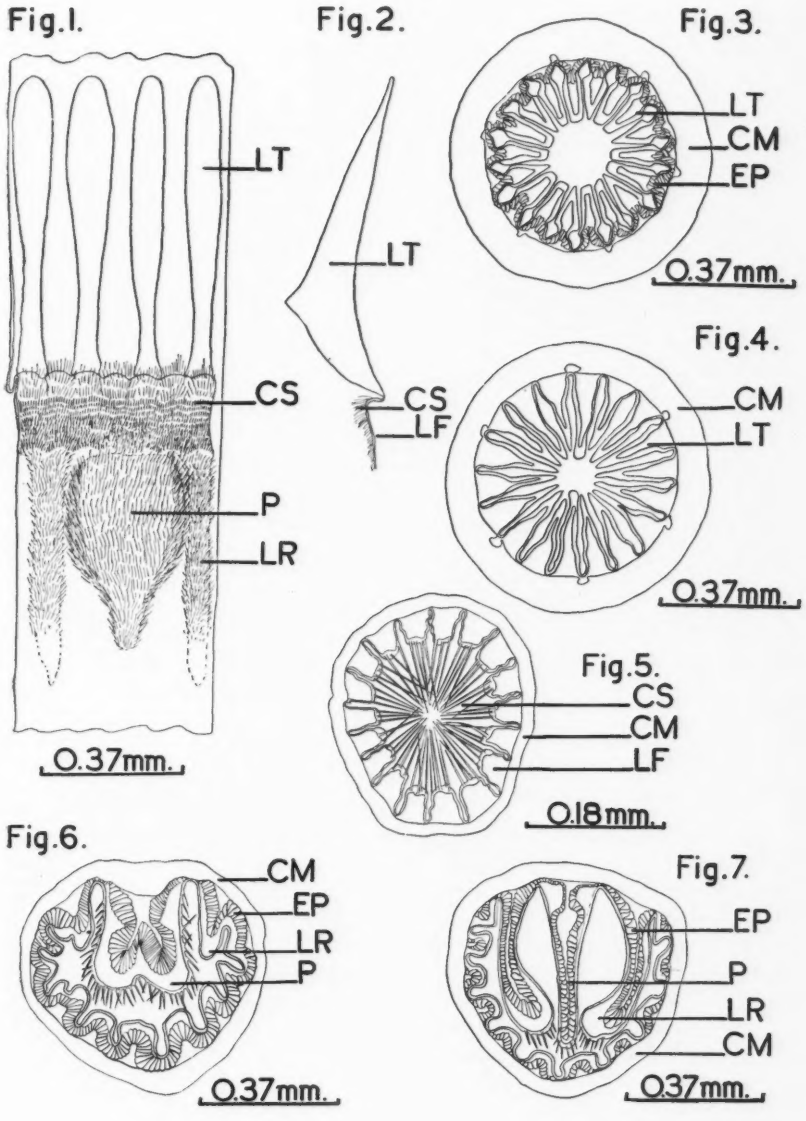
Fig.1.
LT
CS
P
LR
0.37mm.
Fig.2.
LT
CS
LF
Fig.3.
LT
CM
EP
0.37mm.
Fig.4.
CM
LT
0.37mm.
Fig.5.
CS
CM
LF
0.18mm.
Fig.6.
CM
EP
LR
P
0.37mm.
Fig.7.
EP
P
LR
CM
0.37mm.

compressor of food. Lloyd (4) states that the larvae of this family do not chew their victims, but grasp them with their forelegs and thrust them bodily into their alimentary cavity. It would consequently be advantageous for the insect to have a well developed proventriculus for chewing or compressing the ingested insect.

Acknowledgment

The writer wishes to express his gratitude to Prof. J. D. Detwiler of the University of Western Ontario for his guidance in this work.

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THE EFFECT OF TEMPERATURE ON THE RATE AND VARIATION OF OPERCULAR MOVEMENT IN *FUNDULUS DIAPHANUS DIAPHANUS*[1]

By Benjamin N. Kropp[2]

Abstract

The rates of opercular beat of 16 specimens of *Fundulus diaphanus diaphanus* were recorded over a temperature range from 4.3° to 17.5° C. in order to determine how this respiratory movement varied with temperature and some of the sources of variation in rate. While the rate of beat varies directly as the temperature, over a period of several hours at any constant temperature continuous recordings of the rate show recurring cycles of rise and fall in beat frequency that are chiefly responsible for the scatter of the observations. Both the duration of a cycle and the limits of rise and fall for each cycle are definitely set by the temperature. The possible dependence of these phenomena upon central nervous activity is discussed.

The rate of respiratory movements in poikilothermic animals, and in newborn mammals before the thermoregulatory capacity has been developed, usually varies directly with the environmental temperature (Stier and Pincus (5), Sumner (6)). At any constant temperature the observed rate of movement of operculum, gill, or other organ in such animals varies within limits determined by the particular function and temperature (cf. Fig. 1). In the work here reported the attempt was made to determine how respiratory movements in *Fundulus diaphanus diaphanus*, as measured by the observed rate of beat of the operculum, varied with temperature, and some of the sources of variation in rate.

The animals used in this study were collected during August and September. Each animal was kept in running tap water for at least 24 hr. after entering the laboratory, and before being placed in the thermostat for observation. No selection as to size was subsequently made since in preliminary experiments the opercular response to temperature was found to be independent of the size of the fish. Observations were never made on animals that had been in the laboratory for more than four days. The experimental animals were placed in a Fisher absorption cell suspended in a water-bath thermostat with a slow stream of air, which had been brought to the water-bath temperature, bubbling through the chamber at a constant rate. After each change of temperature at least 20 min. were allowed to permit thermal adaptation before readings were resumed at the new temperature. The use of several stop watches made possible the recording of consecutive beats during the period of observation. The following data are based on observations on 16 animals.

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Contribution from the Department of Anatomy, Queen's University, Kingston, Ont. With financial assistance from the Committee on Scientific Research of Queen's University.

[2] *Assistant Professor of Embryology.*

Stop watch readings of the time for 10 opercular beats were made at six different temperatures from 4.3° to 17.5° C. When the average times of 50 readings at each temperature are plotted against temperature (Fig. 1) the points fall along a straight line although there is a marked and progressive divergence from this relationship at lower temperatures. When the extremes

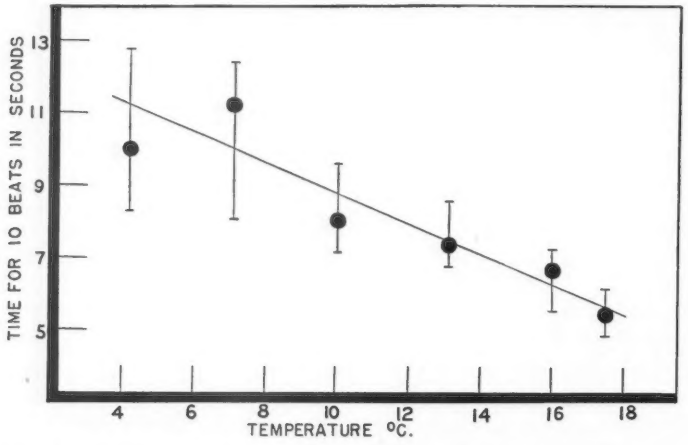

FIG. 1. *Time for 10 beats in seconds at six temperatures from 4.3° to 17.5° C. Average of 50 readings at each temperature. Vertical lines represent limits of variation from each average. Animal No. 2. See text.*

of variation above and below the average for each point recorded are shown on the same curve (vertical lines), it is seen that these variations also tend to become progressively greater at lower temperatures and decrease progressively as the temperature is increased. Many previous investigators have noted the very wide variations that occur in the respiratory rate of cold-blooded animals and have sought to increase the accuracy of their data by recording 'large' numbers of readings, usually 30 to 40, at each temperature. It is apparent that in this particular process the method of averages from relatively few readings at each temperature may indicate trends and may even show occasional extreme rates, but gives limited information regarding the relationship of opercular beat to temperature, and no insight either into the process controlling the opercular beat or the nature and variation of the temperature effect. Therefore, to expand the limits of the method, and in order to include all the extreme rates, readings were made for several thousand consecutive beats over the temperature range under investigation. This was done by continuous stop watch readings of the time for 10 opercular beats, taken with the aid of several stop watches and assistants to record the readings (3). Occasional spontaneous movements prevented obtaining completely successive readings, but except when they were particularly violent these movements did not affect the frequency of opercular beat.

It was found that whenever the temperature of the medium was changed there was an almost immediate rise in the frequency of opercular beat whether the temperature was raised or lowered. There followed a fairly regular decline and rise in the rate of beating, which is illustrated by the data plotted in Fig. 2. The variation or scatter of the points did not occur more or less symmetrically about an average that was characteristic for each temperature. For the data at 17° (Fig. 2, lower curve) there are four definite and successive cycles shown during which the rate of opercular movement gradually declines. The end of each cycle is marked by an abrupt return to a rapid rate and the beginning of a new cycle. In general, the cycles recur more frequently at higher temperatures than at lower ones. Thus, the data at 7° (Fig. 2, upper curve) show but two cycles although in this case the total elapsed time is greater than at the higher temperatures.

The manner of scatter of the observations is well illustrated in Fig. 3. This represents 50 readings at each of the six temperatures indicated on the curve from 5.5° to 17° C. For purposes of comparison these 50 readings were in every case taken at the beginning of a cycle. It will be noted that the scatter of the observations, as indicated by the progressive narrowing of the band enclosing the readings at each temperature, decreases with rising temperature (cf. Fig. 1). This is obvious not only in the observed difference between average frequencies, but also in the decrease of the deviation of the measurements with increasing temperature.

Discussion

Our knowledge of the fluctuations in the underlying process controlling the beat can in most instances be obtained only by assuming that the measured frequencies of beating is an index of these underlying changes. Hence, the frequency of bursts of efferent impulses was assumed to be directly proportional to the velocity of the underlying reactions. The suggestion has been made that periodic variations in the frequency of processes controlling the beat or movement of organs may account for a number of observed differences in frequency of beat at any constant temperature. With regard to the opercular beat of the goldfish Crozier and Stier (4) suggested that the observed variations might be traced to the method of discharge of central nervous impulses controlling the beat. In the case of processes under nervous control it has been possible to measure the fluctuations in the rate of the presumed controlling nerve impulses, and it has in fact been demonstrated (Adrian (1), Adrian and Buytendijk (2)) that nerve impulses may be transmitted regularly along spontaneously discharging nerves from spontaneously discharging centres. In the latter work rhythmical potential changes lasting one to three seconds in the excised brain stem of the goldfish were recorded, and the duration of these potential changes corresponded well with the actual frequency of opercular respiratory movements of the intact fish. The assumption may be made that these centrally originating variations in potential are transmitted as synchronous bursts of efferent impulses to the respiratory musculature.

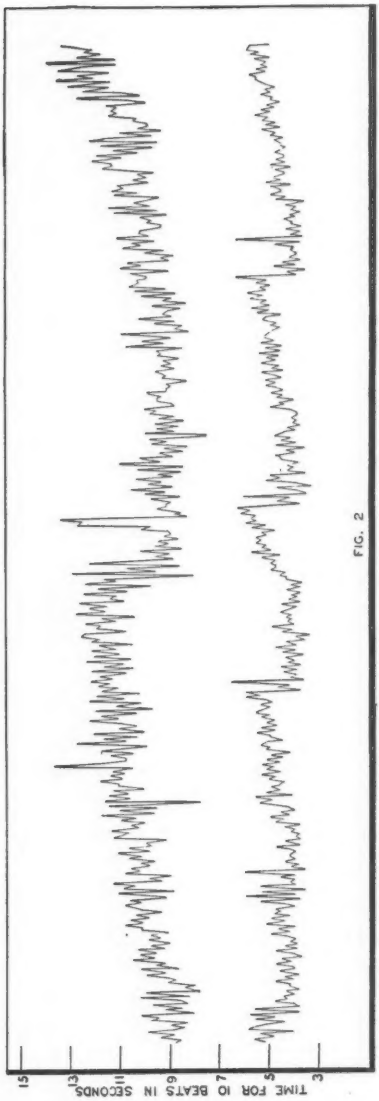

FIG. 2. *Four hundred successive readings (out of several thousand) of time for 10 opercular beats at 7° C. (upper curve) and 17° C. (lower curve). Animal No. 10. See text.*

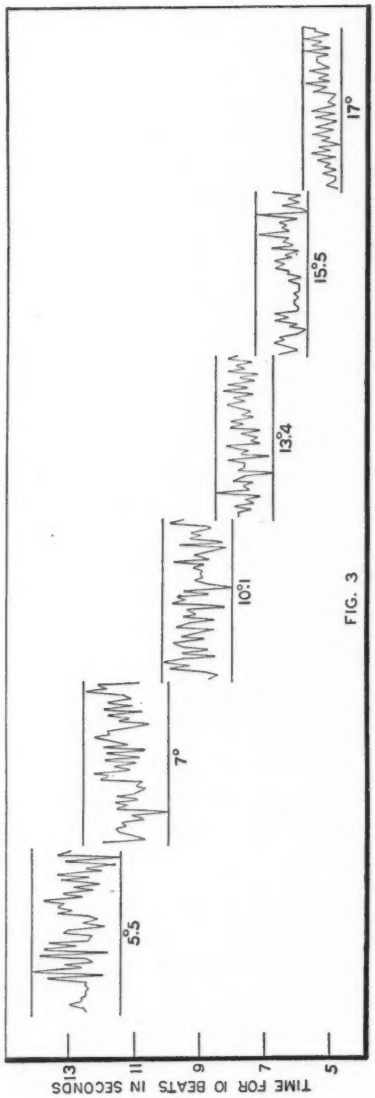

FIG. 3. *Fifty successive readings of time for 10 beats taken at each indicated temperature. Animal No. 6. See text.*

The question arises as to the possible influence on opercular beat of the uncontrolled factor of variation in oxygen consumption, especially since, in studies on *F. parvipinnis*, Wells (7) found the respiratory metabolism to be relatively high at the beginning of his experiments and decreasing with time. This decrease was accompanied by a gradual decrease in oxygen consumption during the first 24 hr. the animals were kept in the laboratory, and thereafter it attained a fairly constant level. Crozier and Stier (4) were unable to find a significant effect upon the breathing frequency of the goldfish after experimentally varying the oxygen concentration from 0.68 to 6.2 cc. per litre. In the present experiments no readings were taken on the opercular beat during the first 24 hr. after the animals were collected, nor were consecutive readings made for as long as a 24 hr. period. However, in observations that lasted as long as six to eight hours on single animals, the gradual decline in rate of opercular beat always ended with an abrupt return to a faster rate and the beginning of a new cycle. There is no reason to believe, therefore, that the gradual slowing of the beat frequency in *F. diaphanus diaphanus* can be accounted for by reduced oxygen consumption, but would seem to be due rather to spontaneous variations of discharge from the respiratory centre.

The possible influence of spontaneous activity on the rate of respiratory movements in the fish is difficult to assess. It occasionally seemed as though the more violent movements may have induced accelerations in rate and the subsequent quiescence was responsible for retardation. But it is more likely that the increase in respiratory rate is of central nervous origin, and the occasional accompanying bodily movements are due to central nervous or external stimulation. Since the described fluctuations in rate, at any temperature, very often take place with very little or no activity of the entire animal it is difficult to see how the activity could be related causatively to the rate.

Acknowledgments

I wish to acknowledge with thanks the assistance in this work of Mr. George H. Clark and Mrs. Laurel C. Kropp.

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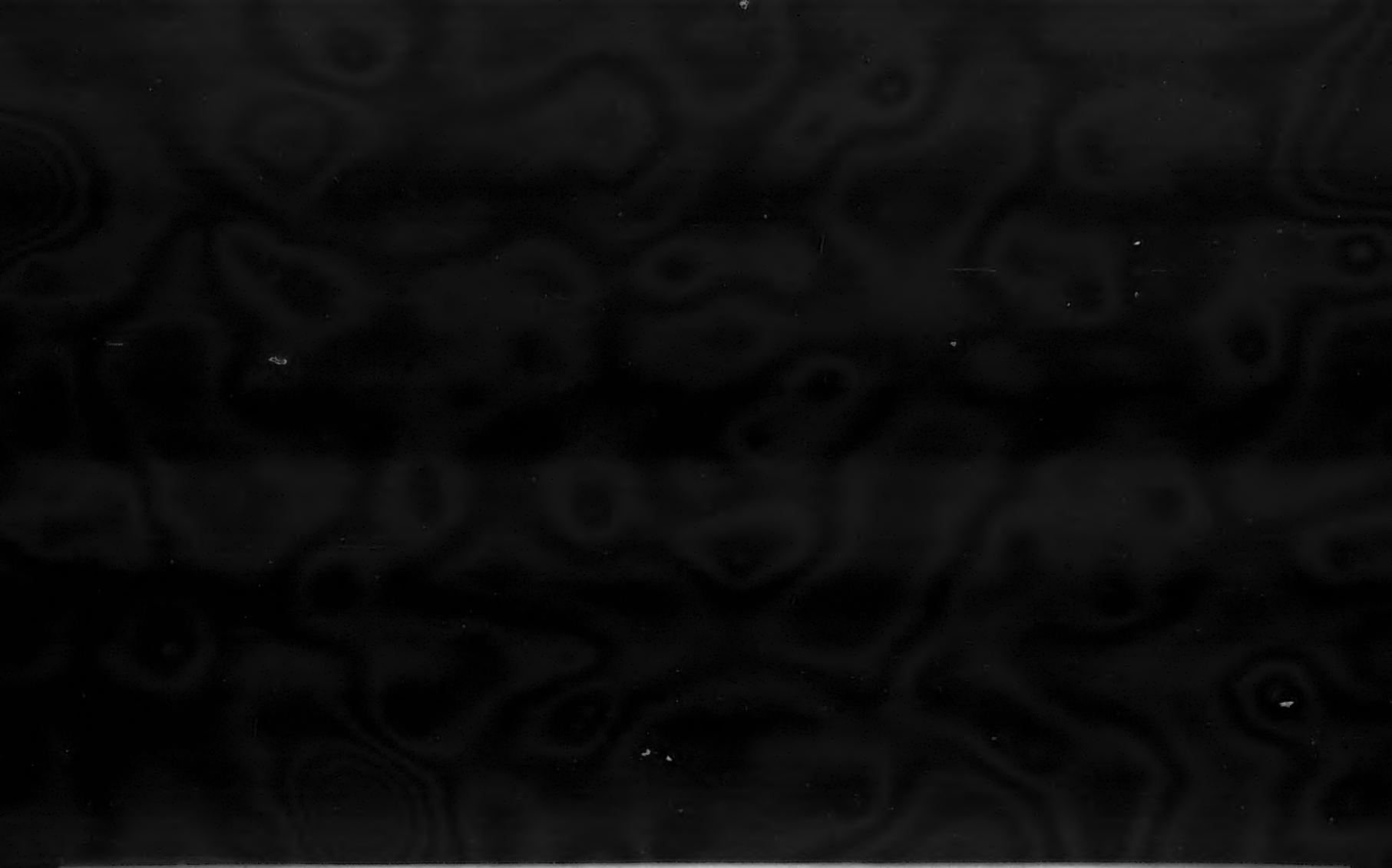

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